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THE NATURE AND INHERITANCE OF MATING TYPES IN EUPLOTES PATELLA[1]

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IN A previous paper (KIMBALL 1939b), the occurrence of six mating types in the ciliate protozoan *Euplotes patella* was reported. These have since been designated by the Roman numerals I to VI. Considerable information which has now been gained about the inheritance and nature of the action of the mating types in this organism will be set forth in the present paper.

The investigations presented here may be divided into three main parts: (1) the method of inheritance of the mating types as determined by appropriate crosses, (2) evidence on the nature of the action of the mating type in bringing about conjugation, (3) an analysis of the exceptional conjugation, which, as pointed out by KIMBALL (1939b), is not infrequent in this species. The results of these investigations indicate that the mating types in *Euplotes patella* are basically dependent upon a relatively simple genetic mechanism. However, the exceptions, which are not uncommon, indicate that there are secondary complications, but these are not thoroughly understood. The inheritance of mating types appears rather different from that found by SONNEBORN (1939) for *Paramecium aurelia* and by JENNINGS (1941) for *P. bursaria*.

METHODS

The methods of culture have been described in a previous paper (KIMBALL 1941). Testing exconjugant lines[2] for mating type was based on the fact that when animals of different mating type are mixed, conjugation ordinarily occurs, but when animals of the same mating type are mixed, conjugation as a rule is not found. Therefore each line of unknown mating type was mixed with lines of each of the six mating types, and the mating

[1] The work for this paper was begun at the Osborn Zoological Laboratory of YALE UNIVERSITY where the author held a Sterling Fellowship. Most of it was carried out later in the Zoological Laboratory of THE JOHNS HOPKINS UNIVERSITY. It was completed at The Biological Laboratory at Cold Spring Harbor.

[2] The word "line" has been used in this paper to designate the descendants of one exconjugant animal. It is thus in many respects equivalent to the word "clone." However, the animals were kept in small mass cultures. Since conjugation was known to occur in these cultures, the original clone was probably shortly converted into a mixture of clones. Thus the word "clone," meaning the descendants of one exconjugant by vegatative reproduction alone, cannot be used for a line of descent of this sort, and the word "line" has been substituted for it. Further discussion will be found in the text.

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type was considered to be that of the known line with which no conjugation occurred. In all cases, an unmixed sample of each line was kept as a control (hereafter called the control) to make certain that conjugation would not have occurred without mixture; and the test was repeated if an appreciable amount of conjugation occurred in the control. From two to three hundred animals of each line were ordinarily used for a mixture. As will be pointed out below, conjugation sometimes occurs in mixtures where it is not expected and sometimes fails to occur where it is expected. Therefore certain arbitrary rules had to be set up in determining the mating type of a line. For this purpose, 20 pairs was taken as an arbitrary boundary line. It represents about one-tenth to one-fifteenth of the number of pairs that would be formed if all the animals had conjugated. When 20 or more pairs occurred in the mixtures with each of the six types, the mating type of the line was not considered as definitely established. When conjugation occurred in the mixtures with each of the mating types but with fewer than 20 pairs in one of them, the mating type was taken as that of the type with which least conjugation occurred only if on several repetitions of this mixture no pairs were formed. Otherwise the mating type of the line was considered questionable. When less than 20 pairs or no pairs occurred in mixtures with more than one mating type, the mating type of the line was considered established only if on repetition of these mixtures conjugation was not observed to occur with one of the types and more than 20 pairs were formed with the others. Mixtures yielding about the same number of pairs as the controls were disregarded and later repeated.

The methods of obtaining the various sorts of matings by use of double animals have been described in an earlier paper (KIMBALL 1941). If double animals of a known mating type and with but one micronucleus are mixed with single animals of a different mating type, conjugation occurs in the mixture. A number of different kinds of conjugant groups are formed of which three were of importance for this investigation. These are pairs composed of two single animals, pairs composed of a single animal conjugating with the micronucleate half of a double animal, and pairs composed of a single animal conjugating with the amicronucleate half of a double animal. The first sort of pair involves conjugation between animals of the same line and so basically of the same mating type. The second sort of pair is a true cross between animals of different mating types. In the third sort of pair, the single animal contributes one of its pronuclei to the double animal but does not receive any pronucleus from it. Presumably, the new macronucleus and micronucleus in the single, following conjugation, are derived from the one haploid pronucleus remaining in the single. Thus the singles from such a mating should not be heterozygous for any genes.

The method of studying the inheritance of mating type following conjugation was, then, to isolate in depression slides a number of pairs of the desired sort, one to each depression. After the separation of the conjugants, the two members of a pair were isolated separately in certain experiments; but in most only one member of a pair was kept, the other being discarded. If the anlage of the new macronucleus, visible in the living animal, was not seen, the pair was discarded, since this indicated that the pair had separated without completing conjugation. In case of pairs involving a double animal, the double was discarded in the great majority of cases, since it was more difficult to test the mating type of the doubles than of the singles. Each isolated exconjugant gave rise to a line which was cultivated by the small mass culture technique until its mating type was ascertained or until it was discarded or lost.

INHERITANCE OF MATING TYPE FOLLOWING CONJUGATION

One of the major aims of this investigation was to study the inheritance of the mating types following conjugation. Because of the time-consuming process of testing the exconjugant lines for mating type, the investigation was not carried out with so large a number of lines or so great a variety of matings as could be desired. Nevertheless, the results that have been obtained present consistent evidence on the kind of inheritance, and it seems desirable to record them at this time.

Preliminary studies indicated that ordinarily the two members of a conjugant pair gave rise to lines of the same mating type. Out of 69 pairs, 63 gave rise to two lines of the same mating type. With random distribution of the types to the two members of a pair in each of the matings studied, only about 27 such pairs would have occurred. The difference is clearly significant. Similarly COHEN (1934) found that in Euplotes the two members of a pair gave rise to two clones that were alike in regard to body form. He pointed out that this would be expected with genic inheritance since cytological evidence made it likely that the two pronuclei in each conjugant were genotypically alike. He also found exceptions to the rule that the two members of a pair give rise to like clones and showed that this would be expected if the nuclear processes did not always occur in the same way. Thus there is good reason to believe that the mating types are determined by gene action.

Since it was found in these investigations that the lines from the two members of a pair were generally of the same type, the study of the inheritance was carried out using only one member of a pair. In those cases in which the lines from two members were studied only one is recorded.

The results of the various matings are given in table 1. The lines recorded in the column under the question mark are those for which ade-

quate tests were not obtained under the rules set forth in the section on methods. None of these lines gave indication of belonging to any of the six mating types other than those expected in the mating in question. Almost all of them seemed to belong to one of the expected types. In most cases only one mating of a particular sort was carried out. In the case of I×I, I×II, and VI×VI, two independent matings were made for each, though most of the data comes from one of these matings.

If the matings between animals of the same line (basically of the same mating type) are considered first, it can be seen that the mating types can

TABLE 1

The results of the various matings that were made. The number of exconjugant lines of each mating type that were obtained from the matings is indicated. Only one line is recorded for each conjugant pair. The symbol ? means that the type of the lines in question could not be ascertained with certainty. The two matings in which one type is enclosed in parentheses are cases in which single animals of one type conjugated with the amicronucleate half of double animals whose type is indicated in parentheses.

	MATING TYPES OF THE PROGENY							
MATINGS	I	II	III	IV	V	VI	?	TOTALS
I×I	32	0	0	14	1	20	2	69
II×II	0	50	16	19	0	0	9	94
IV×IV	0	0	0	113	0	0	11	124
V×V	0	0	3	0	24	8	29	64
VI×VI	0	0	0	0	0	115	24	139
IV×VI	64	0	0	5	0	5	8	82
I×II	7	15	0	22	13	0	8	65
I×(II)	0	0	0	8	1	8	2	19
VI×(IV)	0	0	0	0	0	19	5	24

be put into two categories depending on whether they produced exconjugant lines only of the same type as themselves or of different types. Types IV and VI fall in the first category, types I, II, and V in the second, and type III has not been tested. It is to be noted particularly that, with the exception of type I, types of the second category produced exconjugant lines of which about half are of the same type as the parent line and about a quarter each are of two other types. Type I falls in line with the others if the one type V line is disregarded. This one type V line could well have resulted from an experimental error. The I×I pairs were obtained from a mixture of type I single animals and type II double animals. Double animals occasionally produce single animals (KIMBALL 1941). While every effort was made to remove all the singles from the doubles before mixture, a few may have been overlooked. If such a type II single animal had conjugated with a type I single animal, a type V line could have been produced as may be seen from the results of conjugation between types I and II presented in table 1.

The results of these matings can be explained if it is assumed that types I, II, and V are determined by the different heterozygous combinations of three allelic genes which in their homozygous state determine types III, IV, and VI, respectively. On this hypothesis, type I has one allele in common with type II, and this allele is homozygous in type IV. It has one allele in common with type V, and this is homozygous in type VI. Type II has one allele in common with type V, and this is homozygous in type III. These alleles will be designated, respectively mt^1, mt^2, and mt^3. The genotypes of the various mating types may be designated as follows: type I, mt^1mt^2; type II, mt^1mt^3; type III, mt^3mt^3; type IV, mt^1mt^1; type V, mt^2mt^3; type VI, mt^2mt^2.

It is to be noted that the mating III×III, which would prove that type III is homozygous, has not yet been made. However, the fact that type III has appeared as expected in the progeny of II×II and V×V and not in any of the other matings makes it probable that it is the third homozygote.

If the hypothesis is correct, the mating of two of the homozygous types should result in progeny all of the mating type determined by the heterozygous combination of these alleles. One such mating, type IV with type VI, has been made. Of the progeny, the great majority were of the expected type I; but five were of type IV and five of type VI. It seems quite possible that these last two types resulted from a failure of syngamy in the conjugants either through failure of exchange of pronuclei to take place or through failure of pronuclear fusion after exchange. Aberrant behavior of the pronuclei has been reported by CHEN (1940) for *Paramecium bursaria*, and KIMBALL (1941) has presented evidence that the pronuclei do not always behave normally in *Euplotes patella*. This explanation is made more probable by the fact that in this mating the only two exconjugants in which cytological evidence of abnormal nuclear behavior was obtained gave rise to two of the five type VI lines.

The mating of two heterozygous types should yield progeny in a 1:1:1:1 ratio. The mating types should be the two parent types, the type determined by the homozygous condition of the allele the two have in common, and the third heterozygous type. Only one mating of this sort has been carried out, that of type I with type II. The progeny are of the four expected types. The question of deviation from the expected 1:1:1:1 ratio will be dealt with below.

The last two matings in the table involve exconjugant lines from a mating of single animals of a certain type and the amicronucleate half of double animals of another type. The type of the double animal is indicated in parentheses, since its nucleus is not involved in this mating (see section on methods). Only the clones descended from the single animals were studied in these cases. As mentioned in the section on methods, the

nuclear complexes of the lines descended from such singles should be derived from one of their haploid pronuclei. As a result, the mating types of such lines should be one of the possible homozygous mating types. The results fall in line if the one type V clone produced in the I×(II) mating is disregarded. KIMBALL (1941) pointed out certain sources of error in the methods of ascertaining that the single animal conjugated with the amicronucleate half of the double animal. It seems likely that the one exception represents such an error; for if the type I single animal actually conjugated with the micronucleate half of the type II double animal, the exconjugant line from the single could well have been type V.

In several of the matings a certain ratio between the various classes of progeny would be expected on the hypothesis presented in this paper. Are these ratios realized? To test this the χ^2 test was applied to the results of these matings, excluding the one type V clone in the case of the I×I mating. In the case of the matings I×I and II×II, the fit to a 1:2:1 ratio was satisfactory. χ^2 was 1.15 and 2.84, respectively, and with n equal to two, P was .57 and .25. However, in the case of V×V and I×II, the fit was not particularly satisfactory. χ^2 was 6.26 and 8.05, respectively; and with n equal to two or to three, P was .045 and .046. With the small numbers involved it cannot be said that this rules out the possibility that these are merely random deviations from the expected ratios. Nevertheless, it is possible that some systematic deviations are involved. It is to be noted that in the case of the V×V mating considerable difficulty was experienced in obtaining satisfactory tests for mating type. Part of this difficulty may be traced to the type III tester. Conjugant pairs were very frequent in the unmixed samples of this tester at this time. As a consequence many tests for type III were of necessity discarded. It is likely that a more satisfactory ratio would have been obtained if some of the probable type III lines had not been excluded on this account. No such explanation is available in the case of the I×II mating. Perhaps in this case some systematic deviation from expectation is involved. Despite the lack of a close agreement with the expected ratio in all cases, it is felt that the appearance of the expected classes and the agreement with expectation found in those cases in which the numbers were largest and conditions of testing most satisfactory support the hypothesis.

It cannot be claimed that the hypothesis of mating type determination presented here is completely established by the breeding experiments, and it is planned to continue the work and present further evidence when it is available. Nevertheless, the results of the matings and results of other types of investigation to be presented below are very consistently explained by it. The most serious deviations may be explained by certain very possible sources of error in the methods used to ascertain the origin

of the nuclei in the various exconjugant lines. It should be stated that from time to time the mating types of exconjugant lines from mixtures of single animals of different mating type have been tested. The results are consistent with the hypothesis; but since it is not possible to ascertain the exact ancestry of the exconjugants from such mixtures, the results are not presented in detail.

THE SPECIFIC ACTION OF ANIMAL-FREE FLUID IN INDUCING CONJUGATION

KIMBALL (1939b) has reported that animal-free fluid taken from cultures of one mating type may induce conjugation among animals of another type. An investigation of the results of mixing fluid from each of the six types with animals of each has since been made. To do this, fluid was taken from a culture of a particular mating type, and all the animals were removed under the microscope with a fine pipette. To this fluid, animals (and fluid) of the desired sort were added. This was repeated for each mixture set up. Controls consisting of animals and fluid from the same culture were invariably used. The amount of fluid used was held as constant as possible, but considerable variation did occur.

Table 2 shows what was found. Fluid from any one of the heterozygous types induces conjugation among animals of any of the other five types. However, fluid from a homozygous type induces conjugation only when mixed with animals of a type with which it does not have an allele in common. All of these mixtures have been repeated 15 to 30 times. However, it should be emphasized that exceptions to the system presented in table 2 do occur. Some of these exceptions involved conjugation of animals when mixed with fluid from the same culture as themselves. The possible causes

TABLE 2

The results of mixing culture fluid, from which all animals have been removed, with animals of various mating types. The type of the animals is indicated across the top of the table, the type of the culture from which the fluid was obtained down the side of the table. The plus sign indicates that conjugation ordinarily occurred, the minus sign that it ordinarily did not. The postulated genotypes of the various mating types are indicated in the first column.

GENOTYPE	FLUID	ANIMALS					
		I	II	III	IV	V	VI
mt^1mt^2	I	−	+	+	+	+	+
mt^1mt^3	II	+	−	+	+	+	+
mt^2mt^3	III	+	−	−	+	−	+
mt^1mt^1	IV	−	−	+	−	+	+
mt^2mt^3	V	+	+	+	+	−	+
mt^2mt^2	VI	−	+	+	+	−	−

of this phenomenon will be discussed under the head of conjugation between animals of the same line. In other cases, conjugation failed to occur in mixtures where it was expected. On repetition of these mixtures, it always occurred. The third group of exceptions involved conjugation in mixtures in which it was not expected. A later section discussing the conjugation in mixtures of animals of lines presumably of the same type applies to these exceptions also. It is hoped that a careful study of such cases, using fluids of known sorts, will aid in investigating further the basis of these various sorts of exceptions. A more detailed account will be reserved until such investigations have been made.

The results so far obtained can be tentatively explained if it is assumed that each of the three alleles of the mating type gene is responsible for the production by the animal of a particular substance which gets into the culture fluid and induces conjugation only in animals which do not produce that substance. Thus animals of a heterozygous type would produce two substances and so would be induced to conjugate only by the third one. Animals of a homozygous type would produce only one substance and so would be induced to conjugate by either of the other two. Fluid from a heterozygous type would induce conjugation in any other type, but fluid from a homozygous type would contain but one substance and so would induce conjugation only in those types lacking the allele in question.

AMOUNT OF CONJUGATION IN MIXTURES OF THE VARIOUS MATING TYPES

It was noticed early in the work that the number of pairs formed in mixtures of certain mating types was generally fewer than the number formed in those of others. There was considerable overlap and variation so that large numbers of pairs were formed in mixtures in which small numbers were more common and small numbers in mixtures in which large numbers were the rule. Nevertheless, in the long run, smaller numbers of pairs were more regularly found in certain mixtures than in others.

A demonstration in numerical form of this difference by counting the number of pairs formed in each mixture was not practicable, since in many cases the number of pairs formed was too large to obtain more than a qualitative estimate. A fairly accurate measurement was obtained in an indirect manner by comparing for the different combinations the percentage frequency of mixtures in which 20 pairs or less were formed. This method is based on the assumption that the probability of only 20 pairs or less occurring would be greater in combinations in which fewer pairs were formed on the average. Table 3 presents the results obtained. It shows for each combination of mating types the number of mixtures that

were made and the percentage of these in which 20 pairs or fewer were found.

TABLE 3

The occurrence of a total of 20 pairs or less in mixtures of different mating types. The particular combination of mating types is given in the first column, the number of mixtures of this sort that were made is given in the second, and the percentage of these in which 20 pairs or less were found in the last.

COMBINATION	NO. OF MIXTURES	PERCENTAGE OF MIXTURES WITH 20 PAIRS OR LESS
I×II	446	1.79%
I×III	254	2.54
I×IV	785	16.05
I×V	289	4.15
I×VI	773	24.45
II×III	340	19.12
II×IV	709	12.27
II×V	224	1.79
II×VI	408	0.74
III×IV	458	4.80
III×V	124	30.65
III×VI	297	0.67
IV×V	431	3.02
IV×VI	660	2.73
V×VI	430	23.72

It may be seen that the percentage of such cases is high in the combinations I×IV, I×VI, II×III, II×IV, V×III, and V×VI. These results confirm the impression obtained during the course of the experiments. The connection with the genetics of the mating types is striking. In each case, a high percentage with 20 pairs or fewer occurred for mixtures between a heterozygous type and one of the types homozygous for an allele found in the heterozygote. The occurrence of smaller amounts of conjugation in these mixtures is readily explained from a knowledge of the action of the fluids. In a mixture of a heterozygous type and a homozygous type with which it has an allele in common, conjugation would be expected to be induced between animals of the homozygous type but not between those of the heterozygous type. Thus a smaller number of pairs would be formed in such mixtures than in those in which both types were induced to conjugate. The rather considerable variability within the low and high percentage groups remains unexplained.

EXCEPTIONAL CONJUGATION

Conjugation within an exconjugant line

As has been said, conjugation sometimes occurs in unmixed samples of one exconjugant line. In such cases, the number of pairs was generally

small but occasionally was rather large—in a few cases almost all the animals formed pairs. Of a total of 2,882 unmixed samples, conjugation was found in 322. In 233 of these, the number of pairs that were formed was fewer than 20; but in the remaining 89 more than 20 pairs were found. There was some indication that conjugation was more frequent in samples of lines which had been kept a long time following the conjugation at which they arose, but this matter needs further investigation.

There are a number of possible explanations for this conjugation between unmixed animals of the same line. In the first place, it was reported by SONNEBORN (1937) and by KIMBALL (1937) that autogamy (endomixis) in *Paramecium aurelia* very frequently results in a change of mating type. Since the lines of Euplotes were kept as small mass cultures and not as isolation lines, autogamy occurring in part of the animals could conceivably lead to the production of cultures composed of several mating types and so to conjugation within a line. KIMBALL (1939b) has already stated that he has been unable to find any sign of autogamy, endomixis, etc., in *Euplotes patella* and that if such processes did occur, they could not account for at least part of the conjugation between animals of the same line. No evidence that would lead to a change in this opinion has since come to light, although watch has been kept for such nuclear reorganizations.

The fact that the animals were kept as small mass cultures rather than as isolation lines offers another possible explanation of this sort of exceptional conjugation. Each line was started from a single exconjugant animal and so was at first a clone. However, conjugation probably occurred at one time or another in all the lines and converted them into mixtures of clones. As has been shown, conjugation results in changes of mating type when it occurs within types I, II, or V but not when it occurs within types IV or VI and probably not when it occurs within type III. Thus some of the conjugation within lines of the first three types may have been the result of these lines being mixtures of several types as a result of a previous conjugation. This explanation could not hold in the case of lines of types III, IV, and VI; and it seems unlikely that it is an important factor in any, since the percentage of samples in which conjugation occurred was if anything somewhat higher in the case of lines of these latter types (12.0 percent out of 1536 samples as compared to 10.4 percent out of 1236 samples).

It is also possible that in setting up mixtures and controls, some animals accidently became transferred from a mixture to a control by splashing. Tests for splashing contamination were made by placing slides with culture fluid but no animals near the slides in which the mixtures were being made. In no case were any animals found in these slides. Though this

source of error cannot be completely disregarded, it probably was not a major factor.

These considerations lead us to the possibility that in Euplotes the mating types may not be inherited unchanged during vegetative reproduction. KIMBALL (1939a) has shown that changes during vegetative reproduction do occur regularly in certain exceptional clones of *Paramecium aurelia*, although in the great majority of the clones no change occurs. In Euplotes, on the other hand, such "unstable" clones may be the rule. However, if this is the case, the mating type must change only for a short time; for whenever the two members of a conjugant pair from an unmixed sample have been separated before any nuclear change occurred, the cultures from them have been found to be the same type as each other and as the line from which they came. Thus change of mating type may occur during vegetative reproduction, but if it does it is only temporary.

One further possibility to be considered is that in Euplotes conjugation might occur between animals of the same mating type. In Paramecium, all evidence shows that conjugation occurs only between animals of different mating types. In Euplotes, KIMBALL (1939b, 1941) has shown that in mixtures between lines of different mating type conjugation occurs between animals of the same line as well as between animals of different lines. The simplest interpretation of this would be that conjugation can occur between animals of the same mating type, though it is not possible to say that some animals of a line may not temporarily change type as a result of mixture. Therefore, while it cannot be affirmed for Euplotes that under certain conditions conjugation may occur between animals of the same mating type, the possibility must be born in mind.

It may be concluded that the main cause of conjugation within a line may be either change of mating type during vegetative reproduction or conjugation between animals of the same mating type. No way of deciding between these two is evident to the writer.

It should be pointed out that the mating type of an exconjugant line remains constant for many months, perhaps indefinitely, despite the occurrence of conjugation within it. There have been no cases in which a line has undergone a change of mating type as a whole even after many months of culture. This is remarkable, since conjugation is known to result in the production of clones of different mating types when it occurs within lines of certain of the mating types. The simplest explanation of this stability of the mating type of a line is that the exconjugants are wholly or partially eliminated from the cultures. This seems not too unlikely in view of the facts that the exconjugants do not divide for three or four days after conjugation and that there is considerable mortality among them. Since the lines were maintained by transferring a small number of animals

to fresh culture fluid every eight to ten days, this would put the exconjugants at a distinct disadvantage compared to the non-conjugants. Moreover, the descendants of the exconjugants cannot conjugate for about a month after conjugation (KIMBALL 1939b); and when they are able to conjugate once more, they probably do so quickly if they are of a type type different from the line as a whole. Thus they are once more eliminated from active participation in conjugation. Therefore at any one time most if not all the animals capable of conjugating probably belong to the original mating type. Accordingly, by testing the mating type of an exconjugant line, the mating type of the original clone from which it was derived may be ascertained.

Conjugation within mixtures of lines of the same mating type

Conjugation has also been found in mixtures of animals from lines which were of the same mating type according to most of the tests that were made with them. Conjugation occurred in 601 out of 2,381 mixtures of this sort. In 317 of these cases, pairs were found in the controls. In 215 of them, the number of pairs in the controls was about the same or larger than the number found in the mixtures. In these cases the occurrence of conjugation was apparently independent of whether mixture was made or not. There are 206 other mixtures which probably belong in this category. The controls for these contained no pairs, but there were only ten pairs or less found in the mixtures. This number is so small that the failure to find pairs in the controls could have resulted from differences in the sample of animals used or in the conditions in the mixtures and controls. The variation between control and mixture is illustrated by the fact that in 131 cases conjugation was found in one or both controls but not in the mixtures.

There remain 180 mixtures in which conjugation apparently occurred as a result of the mixture. These mixtures either contained many more pairs than their controls or more than ten pairs when none were found in the latter. This might be because the two cultures used for the mixture were not of the same mating type, but the fact that in almost all cases repeated tests showed that mixture between the cultures in question only occasionally resulted in conjugation, frequently only once, makes this explanation seem unlikely. Moreover, if they were of different types from each other, one in each case must have belonged to a type other than the six so far established, since conjugation regularly occurred in mixtures of each with the five mating types other than the one to which they were suspected to belong.

In order to test the possibility that more than six mating types existed in the available material, experiments were carried out several times. The

procedure was to use a number of lines which had conjugated when mixed with all six mating types but which were suspected to belong to one of these types. Animals from these lines were mixed in all possible combinations with each other, with animals from one or two lines known to belong to the type to which the others were suspected to belong, and with animals from one line known to be of a different type. In all these tests, conjugation was found in some of the mixtures but not in others. In the mixture with the line of different type, conjugation occurred in all cases, and the number of pairs formed was large. In the other mixtures when conjugation occurred at all, the number of pairs was usually, though not always, small. When the tests were repeated using the same lines, conjugation occurred again but, with the exception of the line of different mating type, not always in the same mixtures. This result is in contrast to that obtained when a number of cultures of different types are mixed several separate times. In this case conjugation occurs repeatedly in certain mixtures and never or rarely in others. The lines fall definitely into well marked groups, the mating types, while in the tests described above the lines do not fall into a system. Instead, each line acts as though it were different from every other and acts in different ways at various times so that no systematic grouping can be made. If we assume that the conjugation in these cases is a result of the lines being of different mating types, then each line must be a type of its own, and this type must vary from time to time. Whether individual differences do exist between lines in regard to mating proclivities cannot be decided with the evidence available, but almost all the lines studied so far have fallen more or less clearly into six classes. Conjugation may occur sporadically in mixtures between lines of the same class but occurs quite regularly in mixtures of lines of different classes.

The reason for conjugation within mixtures of lines of the same mating type is not clear at present. Part of it can probably be explained as conjugation which would have occurred whether the lines had been mixed or not. However, it seems doubtful if all the cases come in this category, for conjugation occurred in a number of mixtures whose controls contained far fewer pairs than the mixture or none at all.

Failure of conjugation where it is expected

In a number of cases, when animals from a line were mixed with each of the six mating types, conjugation failed to occur in two or more of the mixtures. However, when repeated tests were made, conjugation occurred in all save one. In a few instances the lines were lost before sufficient repetitions were possible, but there is no reason to believe that they would have continued to fail to conjugate with two or more of the types. Nevertheless, such lines were of necessity considered of doubtful mating type. The failure

of conjugation to occur in mixtures of lines that are of different mating type is possibly dependent upon some environmental conditions which are not fully understood.

DISCUSSION

The investigation of mating type inheritance in Euplotes has yielded evidence for the second gene to be found in the ciliates, the first having been found by SONNEBORN (1939) in *Paramecium aurelia*. It is the first case of multiple alleles observed in these animals. The genetic evidence is perhaps not sufficient by itself to establish beyond doubt this hypothesis of mating type determination. However, when evidence from other sources is taken into account, it seems fairly firmly established. More evidence on several points is desirable and should be obtained before very long.

According to the hypothesis, each mating type allele is responsible for the production by the animal of a particular sort of conjugation-inducing substance which gets into the fluid, and for resistance of the animal to the action of that fluid. On this basis the interactions of the heterozygous mating types may be conceived as the simple combination of the interactions of two homozygous mating types. The case in this respect is quite like those of blood groups in man and self sterility in flowering plants. The question of whether the similarity is farther reaching than this should be left till a more intensive analysis has been made.

Little can be said at present about the nature of the substances produced under the influence of the different alleles. However, it seems highly probable that the differences between these substances are not simply quantitative but are qualitative; otherwise the independent action of the alleles in the heterozygote would be very difficult to explain. This is further shown by the fact that a consistent system of fluid action has been obtained, although in most experiments no serious attempt was made to keep the quantities of fluid constant.

A comparison of mating type determination in Euplotes with that found in the genus Paramecium reveals no very close similarities between the genera. In *P. aurelia*, SONNEBORN (1939) has found just two mating types in any one non-intermating variety. The presence of the dominant allele of a certain gene allows clones to be of either type. Which one they actually are depends upon the macronucleus, but the way in which this structure is caused to determine a specific type is not completely clear. All clones with the homozygous recessive condition of this gene pair are of the same mating type, type I. The homozygous recessive condition resembles the homozygous conditions of the alleles in Euplotes in determining one mating type, but no homologue has been found in Euplotes for the dominant gene of *P. aurelia*.

In *P. bursaria*, JENNINGS (1939, 1941) has found more than two mating types within each of the non-intermating varieties which he has studied. However, the results of the genetic studies published so far do not appear to lend themselves to any very certain interpretation, though genic inheritance in all probability is involved. The results certainly do not resemble those found for Euplotes.

Not only can no clear similarity be pointed out between mating type determination in Paramecium and Euplotes, but the method of action of the types in bringing about conjugation appears different. KIMBALL (1939b) has already reported that the immediate clumping reaction so characteristic of mixtures of different mating types in Paramecium is apparently entirely lacking in Euplotes. Furthermore, fluid from one mating type does not appear to induce conjugation among animals of another in Paramecium. This is brought out particularly well by the fact that in Paramecium. conjugation in mixtures is usually found only between animals of different clones, not between animals of the same clone as in Euplotes.

The discovery of just three alleles of the gene for mating type in Euplotes may have no real significance. All the clones studied so far are descended from two original animals collected from ponds on the campus of the JOHNS HOPKINS UNIVERSITY. These two individuals were of type I and type II, respectively, and on the hypothesis of mating type determination would have had between them the three mating type alleles that have been found and no others. There is no *a priori* reason why additional alleles and therefore more mating types may not be found when time permits the analysis of further material. In Paramecium, on the other hand, a limited number of mating types have been found, the maximum being eight in one of the varieties of *P. bursaria*. There are good reasons to believe that in *P. bursaria* and *P. aurelia*, at least in the varieties studied, all the possible mating types have been found.

The study of mating type determination in *Euplotes patella* has revealed a relatively simple genetic system which may well lend itself to an extensive analysis of the role of the genes in the physiological processes involved. A beginning in this direction has been made through studies of the action of the fluids in inducing conjugation.

SUMMARY

Six mating types have been found in the ciliate protozoan *Euplotes patella*. When animals of one type are mixed with those of another, conjugation generally occurs in the mixture. Ordinarily it does not occur when animals of the same mating type are mixed.

In a study of the inheritance of mating type at conjugation it was found that the two members of a pair gave rise to lines of like type in the majority

of the cases (63 out of 69 pairs). This indicates that genic inheritance is involved.

The results of matings of various sorts may be interpreted on the hypothesis that the six mating types are determined by the six possible combinations of three alleles of a gene for mating type. There are three types, each homozygous for a different allele, and three heterozygous types, each with a different combination of alleles.

Animal-free fluid from a culture of one mating type induces conjugation among animals of certain other mating types if it is mixed with them.

Fluid from one of the homozygous types induces conjugation only among animals of types which have no allele in common with it. Fluid from one of the heterozygous types induces conjugation among animals of any other type than its own.

Exceptions to this general scheme are found and may be considered in the same light as the exceptions to the general mating type scheme.

The facts may be explained if it is assumed that each allele is responsible for the production of a specific substance by the animal. Animals are assumed to be induced to conjugate only by those substances which they do not produce.

In line with these findings is the fact that in mixtures between a heterozygous mating type and a homozygous one with which it has an allele in common fewer pairs are formed on the average than in other mixtures.

The heterozygous phenotype may then be considered as the result of a simple combination of two homozygous phenotypes. The alleles may be considered to act independently in the heterozygote.

This independent action of the alleles in the heterozygote indicates that the conjugation-inducing substances are qualitatively and not simply quantitatively different.

This hypothesis probably covers the basic facts, but there are secondary complications resulting in exceptional conjugation.

These exceptions are of three sorts: conjugation in unmixed samples of animals from the same culture, conjugation in mixtures of lines which are probably of the same mating type, and failure of conjugation where it would be expected.

An analysis is presented of the available information on these exceptional occurrences, and possible causes of them are discussed.

A comparison between mating type determination in Euplotes and in Paramecium reveals no close similarities.

These studies represent a beginning of an attempt to analyze the role of genes in the physiological processes involved in conjugation in Euplotes.

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INTERSEX MODIFYING GENES IN WILD STRAINS OF DROSOPHILA MELANOGASTER

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THE sex determining mechanism in diploid *Drosophila melanogaster* is completely stable. No intersexual individuals are produced from hybridizing strains of *D. melanogaster* collected from widely separated geographical localities. In Lymantria, on the contrary, GOLDSCHMIDT (1934) has shown that the potencies of the X chromosome sex gene differs in races from Europe and Japan.

The degree of sexuality in triploid intersexes (2X 3A) of *D. melanogaster* in contrast to the diploid sexes is remarkably sensitive to both genetic and environmental mileu (DOBZHANSKY 1930). DOBZHANSKY found that temperature affected the sex type of intersexes. The same author demonstrated the presence of modifying genes in a triploid strain. By means of several generations of selection, he derived a triploid strain producing predominantly male-like intersexes and also a triploid strain producing predominantly female-like intersexes. DOBZHANSKY located intersex modifying genes in chromosome III capable of shifting the intersex sex type when present in only one dose.

The purpose of the present paper is to determine whether or not different intersex modifying genes located in the X chromosome exist in wild populations of Drosophila collected from various geographical regions.

MATERIAL AND METHODS

The twenty wild stocks used in these experiments and their place of origin are as follows: Amherst, Massachusetts; Cal-c, California; Canton, Ohio; Florida; Formosa, Japan; Lausanne, Switzerland; Seto, Japan; Swedish-b, Sweden; Tuscalosa, Alabama; Urbana, Illinois; Woodbury, New Jersey; Caddo Lake, Texas; Henderson, Texas; La Lajita, Mexico; Lubbock, Texas; Mexico 220.1, Mexico; Uvalde, Texas; Fort Worth, Texas; Dallas, Texas. The first twelve of the stocks were kindly sent to the author by DR. M. DEMEREC of the Department of Genetics, CARNEGIE INSTITUTION OF WASHINGTON; the next six stocks by DR. J. T. PATTERSON, of the UNIVERSITY OF TEXAS. The last two stocks were collected by MISS ROSEMARY CALLAN and MR. JULIAN MATESON, respectively. A description of the origin of the wild stocks obtained from DR. DEMEREC may be found in his paper on the frequency of spontaneous mutations in certain stocks of *D. melanogaster* (DEMEREC 1937). The triploid stock used in the

present study has been inbred for four years by the author. It arose originally from a single female. The triploid stock is homozygous for the recessive X chromosome mutants y^2, yellow body; v, vermilion eyes; and f, forked bristles.

In the first set of experiments, males from a wild stock were mated with $y^2\ v\ f$ triploid virgins. Seven triploids and ten males were used to the bottle. A total of from seven to ten bottles was usually made up. In some cases, all the crosses involving a particular wild stock were made up at one time; in others, crosses were made on two or more dates. A record was kept of the date each bottle was made up. Crosses were conducted in an incubator which, however, varied in temperature from 20°C to 26°C.

Among the progeny of wild males and $y^2\ v\ f$ triploids, both $y^2\ v\ f$ and wild type intersexes are found. The $y^2\ v\ f$ intersexes receive both their two X chromosomes from their triploid mother and a Y chromosome from the wild father. The wild type appearing intersexes receive a $y^2\ v\ f$ X chromosome from the triploid mother and a wild X, bearing the normal allels of $y^2\ v\ f$, from the wild male parent. The triploid mother contributes two sets of autosomes to both $y^2\ v\ f$ and wild type intersexes, and the wild male parent contributes one set of autosomes to both kinds of intersexes. Therefore, the $y^2\ v\ f$ and their wild type sib intersexes differ only with respect to the X chromosome (and Y chromosome), since each type has an equal chance of receiving any two of each three autosomes from the triploid female parent and any one of each pair of autosomes from the wild male parent.

If there are genes present in the wild X chromosome dominant over their alleles in the $y^2\ v\ f$ X chromosome and capable of modifying the sex type of the intersexes, then the proportions of the various sex types of wild type intersexes will differ from those of the $y^2\ v\ f$ intersexes. Modifying genes in the autosomes will have an equal chance of being inherited by wild type intersexes or their $y^2\ v\ f$ sib intersexes. The intersexes were classified according to their degree of sexual expression into six arbitrary groups outlined by DOBZHANSKY and SCHULTZ (1934). These are as follows:

"Class I. Extreme male type intersexes. Genitalia and coloration of the abdomen male. Penis and genital arch symmetrical. Sex combs present. Anal tubercle of male or female type.

"Class II. As above, but penis and genital arch asymmetrical.

"Class III. Intermediate intersexes. Neither male nor female external genitalia present, or genitalia extremely rudimentary. Anal tubercle female. Coloration of the abdomen male. Sex combs present.

"Class IV. As above, but female genitalia present. Vaginal plates asymmetrical.

"Class V. Female type intersexes. Female genitalia present. Vaginal

plates symmetrical. Coloration of the abdomen female (rarely intermediate). Sex combs present on at least one leg.

"Class VI. Extreme female type intersexes. As above, but sex combs absent on both legs and coloration of the abdomen female." In the present experiment no extreme female type VI intersexes were found. DOBZHANSKY and SCHULTZ (1934) state that these type VI intersexes are "practically never found in the absence of duplications." Intersexes are always sterile.

Owing to the marked influence of temperature differences upon the sex type of triploid intersexes, homogeneity tests to be described later were made to measure environmental effects on intersexes hatching in different bottles of the same cross. When environmental effects were excluded, the proportion of wild type intersexes falling into the five arbitrary groups was compared with the proportion of $y^2\ v\ f$ sib intersexes. Since these two kinds of intersexes hatched in the same bottles, their environment was the same. Any significant difference in the two proportions must be due to modifying genes in the wild X chromosome introduced by the male parent which are dominant over the intersex modifying genes located in the $y^2\ v\ f$ X chromosome coming from the triploid female parent.

The second set of experiments was originally designed as a check on possible intersex modifying genes located in the Y chromosome introduced by the wild type male parent. For reasons to be given later, the following experiments are not an accurate check on the question of the presence of such Y chromosome modifiers. In the second set of experiments, wild type females were crossed in mass to $y^2\ v\ f$ males derived from the $y^2\ v\ f$ triploid stock. Then the F_1 males were crossed to $y^2\ v\ f$ triploids. These F_1 males possessed a wild X chromosome, but their wild Y chromosome and half of their autosomes were replaced by the Y and autosomes from the $y^2\ v\ f$ triploid stock. Such wild males are designated as wild male minus Y, indicating the absence of the wild Y, but presence of a Y chromosome from the $y^2\ v\ f$ diploid males which are used in keeping the $y^2\ v\ f$ triploid stock.

EXPERIMENTAL RESULTS

Detection of environmental differences between bottles of the same cross

Before comparing the sex types of wild type and $y^2\ v\ f$ intersexes from a cross of males of a given geographical region with $y^2\ v\ f$ triploids, homogeneity tests must be made comparing sex types of wild type intersexes coming from different bottles of the same cross. If the numbers of wild type intersexes falling into the five sex type groups in one bottle of a given cross are not proportional to (that is, heterogeneous) the numbers of wild type intersexes in another bottle of the same cross made up on the same date or on a different date, then these differences must be due to the environment.

Hence the numbers from these two bottles cannot be added together or pooled. If the numbers of wild type intersexes from two different bottles of the same cross are proportional (that is, homogeneous), these numbers may be pooled. The small differences between the two sets of numbers may be accounted for by chance alone, if the two sets of numbers are homogeneous. The homogeneity tests were made by reference to the chi square distribution. The method developed by BRANDT and SNEDECOR is described in FISHER (1932). Unless the number of wild type intersexes in a certain bottle totaled nine, the count from that bottle was discarded. The total number of such intersexes from a single bottle was generally between 15 and 25. The bottles of a given cross were first compared in pairs; then pooled homogeneous pairs were compared. In this way the numbers of the two series of intersex sex types being compared were usually of the same order of magnitude.

Wild type intersexes from each bottle of a given experimental cross were compared in the inter-bottle homogeneity tests rather than $y^2\ v\ f$ intersexes because the former intersexes are much more numerous. This is due to the preponderance of 1X 2A and 2X 1A gametes among the eggs of triploids over the 2X 2A and 1X 1A gametes as a consequence of non-random disjunction of chromosomes at meiosis (BEADLE 1935).

Table 1 is a summary of the results of inter-bottle homogeneity tests of wild type intersexes ($y^2\ v\ f/+$) from a cross of wild type males and $y^2\ v\ f$ triploid females. The first column of table 1 gives the original source (geographical region) of the stock from which the male parents were taken. The second column gives the series number of the experimental cross. A single series number was given to all culture bottles of a given cross made up on the same date. The third column gives the number of bottles counted in each series. The largest number of homogeneous bottles in each series is listed in column four of table 1. In column five is listed the largest number of homogeneous bottles from all series. For example, the four bottles of series one, Caddo Lake males crossed with $y^2\ v\ f$ triploids, were homogeneous with respect to wild type intersexes as were the five bottles of series two. Moreover, column five shows that the five bottles of series two were homogeneous with the four bottles of series one.

A similar procedure was used in testing the homogeneity of wild type intersexes arising in different bottles of the same cross for the second set of experiments. Table 2 is a summary of inter-bottle homogeneity tests of wild type intersexes ($y^2\ v\ f/+$) from a cross of wild male minus the wild Y (wild male $-$ Y) and $y^2\ v\ f$ triploid females. Table 2 is arranged in a similar manner to table 1. The main difference between the two sets of experiments is that in the second set, the number of bottles in each series (that is, bottles made up on the same date) is larger, and consequently the number of series is smaller.

TABLE 1

Inter-bottle homogeneity tests of wild type intersexes produced from a cross of wild type males with y^2 v f triploid females.

MALE PARENT	SERIES NUMBER	NUMBER OF BOTTLES MADE UP ON ONE DATE	NUMBER OF BOTTLES HOMOGENEOUS WITHIN A SERIES	NUMBER OF BOTTLES HOMOGENEOUS BETWEEN SERIES
Amherst	1	3	3	
Amherst	2	3	2	
Caddo Lake	1	4	4	
Caddo Lake	2	5	5	9
Cal-c	1	6	4	
Cal-c	2	3	3	
Cal-c	3	3	3	
Canton	1	2	2	
Canton	2	2	2	
Canton	3	3	3	
Canton	4	2	2	9
Dallas	1	3	3	
Dallas	2	2	2	
Dallas	3	3	2	7
Florida	1	6	6	
Florida	2	5	5	11
Formosa	1	5	5	
Fort Worth	1	7	7	
Henderson	1	2	2	
Henderson	2	1	1	
Henderson	3	6	5	8
Kyoto	1	7	7	
La Lajita	1	6	5	
Lausanne	1	4	4	
Lausanne	2	6	4	8
Lubbock	1	1	1	
Lubbock	2	6	6	
Lubbock	3	3	3	10
Mexico 220.1	1	3	3	
Mexico 220.1	2	1	1	4
Seto	1	1	1	
Seto	2	8	5	6
Swedish-b	1	3	3	
Swedish-b	2	2	2	5
Swedish-b	3	5	5	
Swedish-b	4	6	6	
Tuscalosa	1	7	7	
Urbana	1	2	2	
Urbana	2	2	2	4
Urbana	3	8	7	
Uvalde	1	4	3	
Uvalde	2	6	4	
Uvalde	3a		4	
Uvalde	3b	8	4	
Woodbury	1	7	6	
Woodbury	2	3	3	9

Table 1 and table 2 illustrate the effect of the environment, presumably temperature differences, upon the sex types of wild type intersexes arising from both bottles made up on the same date (belonging to the same series) and also wild type intersexes arising from bottles made up on different dates

TABLE 2

Inter-bottle homogeneity tests of wild type intersexes produced from a cross of wild male − Y with y^2 v f triploid females.

MALE PARENT	SERIES NUMBER	NUMBER OF BOTTLES MADE UP ON ONE DATE	NUMBER OF BOTTLES HOMOGENEOUS WITHIN A SERIES	NUMBER OF BOTTLES HOMOGENEOUS BETWEEN SERIES
Amherst − Y	1	6	6	
Caddo Lake − Y	1	7	6	
Cal-c − Y	1	9	5	
Canton − Y	1	8	8	
Dallas − Y	1	6	4	
Florida − Y	1	4	4	
Florida − Y	2	4	3	
Formosa − Y	1a		4	
Formosa − Y	1b	6	2	
Ft. Worth − Y	1	5	5	
Ft. Worth − Y	2	4	3	8
Henderson − Y	1	6	6	
Kyoto − Y	1	7	7	
La Lajita − Y	1a		4	
La Lajita-Y	1b	6	2	
Lausanne-Y	1	4	4	
Lubbock − Y	1	3	3	
Lubbock − Y	2	8	7	
Mexico 220.1 − Y	1	4	4	
Seto − Y	1	7	6	
Swedish-b − Y	1	4	3	
Swedish-b − Y	2	4	3	4
Tuscalosa − Y	1	4	3	
Tuscalosa − Y	2	4	4	7
Urbana − Y	1	3	3	
Urbana − Y	2a		4	
Urbana − Y	2b	6	2	
Uvalde − Y	1a		4	
Uvalde − Y	1b	6	2	
Woodbury − Y	1a		5	
Woodbury − Y	1b	7	2	

(belonging to different series). In general, most bottles made up on the same date yielded wild type intersexes of homogeneous sex types with one another. Tables 1 and 2 show that usually not more than one or two bottles, if any at all, out of six bottles made up on the same date yielded wild type intersexes heterogeneous with wild type intersexes coming from other

bottles of the same series. Wild type intersexes coming from different series of the same cross were more apt to be heterogeneous, as would be expected, since there would be more chance for temperature differences to occur.

Comparison of pooled wild type and y^2 v f sib intersexes

The pooled numbers of homogeneous wild type intersexes and the pooled numbers of their sib y^2 *v f* intersexes arising from a cross of wild male with y^2 *v f* triploid female are given in table 3. The results of homogeneity tests by the chi square method between the numbers of wild type intersexes and the numbers of y^2 *v f* intesexes are likewise given in table 3. The value of χ^2 is listed; f, the number of degrees of freedom; P, the probability that the two series of numbers compared differ, due to chance alone. P below 0.05 is used as the arbitrary limit of homogeneity, following R. A. FISHER.

Table 4 is constructed in a similar way to table 3. Table 4 compares pooled wild type and y^2 *v f* intersexes coming from the cross of wild male minus Y with y^2 *v f* triploid females.

Table 3 shows that 12 of the 20 different wild stocks gave wild type and y^2 *v f* sib intersexes homogeneous with respect to their sex types. These stocks were as follows: Amherst, Cal-c, Canton, Ft. Worth, Kyoto, La Lajita, Lausanne, Lubbock, Mexico 220.1, Seto, Urbana, Woodbury. Six of the 20 different wild stocks produced wild type intersexes heterogeneous with their sib y^2 *v f* intersexes. These included Dallas, Florida, Formosa, Henderson, Tuscalosa, and Caddo Lake.

In two cases, Swedish-b and Uvalde, conflicting results were obtained from different series of the same cross. The first series of Swedish-b males crossed with y^2 *v f* triploids resulted in heterogeneous wild type and y^2 *v f* intersexes. That is, the probability that the proportion of wild type intersexes falling into the five sex types differed from the proportions of their sib y^2 *v f* intersexes was between 0.05 and 0.01. This falls just below P equals 0.05, the arbitrary limit of homogeneity, and is consequently a borderline case. Two further series of Swedish-b males crossed with y^2 *v f* triploids yielded homogeneous wild type and y^2 *v f* intersexes.

Similarly, the first series of Uvalde males crossed with y^2 *v f* triploids gave homogeneous wild type and y^2 *v f* intersexes. On the other hand, three subsequent series of the same cross produced heterogeneous wild type and y^2 *v f* intersexes. Possibly the Swedish-b and Uvalde stocks may have contained X chromosomes, part of which possessed intersex modifying genes of similar potency to those in the y^2 *v f* X chromosome, while other X chromosomes of Swedish-b and Uvalde contained intersex modifying genes capable of altering the proportion of sex types in wild type intersexes from those in their y^2 *v f* sib intersexes. In 21 other cases from tables 3 and 4,

TABLE 3

Comparison of the sex types of y^2 v f/+ intersexes and their y^2 v f/y^2 v f sib intersexes from a cross of wild type males with y^2 v f triploid females.

MALE PARENT	SERIES NUMBER	y^2 v f/+ INTERSEX						y^2 v f/y^2 v f INTERSEX						x^2	f	P	HOMOGENEITY
		I	II	III	IV	V	TOTAL	I	II	III	IV	V	TOTAL				
Amherst	1	9	26	27	21	5	88	16	15	21	25	2	79	6.8287	4	0.2–0.1	hom.
Amherst	2	20	3	25	16	2	66	9	4	4	12	1	30	8.0619	4	0.1–0.05	hom.
Caddo Lake	1+2	83	37	56	68	11	255	32	25	35	51	14	157	9.8169	4	0.05–0.02	het.
Cal-c	1+3	22	29	32	101	8	192	3	8	8	18	3	40	2.3406	4	0.7–0.5	hom.
Canton	1+2+3+4	12	29	28	140	32	241	8	15	16	68	13	120	0.2290	4	0.99	hom.
Dallas	1+2+3	73	47	46	30	2	198	22	13	36	20	5	96	17.9208	4	<0.01	het.
Florida	1+2	80	62	75	126	18	361	11	12	13	42	12	90	15.8700	4	<0.01	het.
Formosa	1	45	19	61	1	0	126	17	19	50	4	0	90	9.8058	3	0.05–0.02	het.
Ft. Worth	1	40	16	40	22	1	119	10	7	20	13	0	50	3.9981	4	0.5–0.3	hom.
Henderson	1+2+3	108	29	40	30	0	207	27	23	25	19	4	98	23.2336	4	<0.01	het.
Kyoto	1	10	18	28	85	54	195	9	3	9	39	19	79	6.4079	4	0.2–0.1	hom.
La Lajita	1	35	30	32	26	2	125	11	12	18	22	1	64	5.7304	4	0.3–0.2	hom.
Lausanne	1+2	11	12	31	87	18	159	2	7	11	43	8	71	2.5061	4	0.7–0.5	hom.
Lubbock	1+2+3	72	28	35	50	9	194	29	18	24	28	6	105	6.8798	4	0.2–0.1	hom.
Mexico	220.1 1+2	11	3	24	28	2	68	5	10	20	21	1	57	6.7996	4	0.2–0.1	hom.
Seto	1+2	24	18	45	41	0	128	14	16	18	29	2	79	7.1810	4	0.2–0.1	hom.
Swedish-b	1	73	22	23	10	1	129	23	7	12	12	2	56	10.6192	4	0.05–0.02	het.
Swedish-b	2	49	23	12	21	1	106	12	8	6	5	0	31	2.1251	4	0.8–0.7	hom.
Swedish-b	3	22	27	22	17	1	89	12	12	17	11	0	52	2.0704	4	0.8–0.7	hom.
Tuscalosa	1	14	31	27	83	11	166	5	7	9	53	22	96	21.5545	4	<0.01	het.
Urbana	1+2	27	13	23	79	12	154	8	6	7	50	12	83	7.3326	4	0.2–0.1	hom.
Urbana	3	23	43	78	88	4	236	3	9	27	41	4	84	9.4411	4	0.1–0.05	hom.
Uvalde	1	55	18	12	6	1	92	40	20	15	13	1	89	5.3381	4	0.3–0.2	hom.
Uvalde	2	81	37	33	20	2	173	18	9	9	21	5	62	25.3979	4	<0.01	het.
Uvalde	3a	55	48	31	16	0	150	15	7	9	13	4	48	23.5369	4	<0.01	het.
Uvalde	3b	37	8	19	14	0	78	0	3	3	13	1	20	27.1236	4	<0.01	het.
Woodbury	1+2	51	32	30	63	7	183	8	8	7	19	3	45	2.7134	4	0.7–0.5	hom.

TABLE 4

Comparison of sex types of y^2 v f/+ intersexes and their y^2 v f/y^2 v f sib intersexes from a cross of y^2 v f triploid females with males carrying a wild X chromosome and a Y chromosome derived from the triploid stock.

MALE PARENT	SERIES NUMBER	y^2 v f/+ INTERSEX						y^2 v f/y^2 v f INTERSEX						χ^2	f	P	HOMO-GENEITY
		I	II	III	IV	V	TOTAL	I	II	III	IV	V	TOTAL				
Amherst−Y	1	17	23	22	57	7	126	5	11	10	22	0	48	3.5305	4	0.5–0.3	hom.
Caddo Lake−Y	1	15	21	20	34	3	93	2	4	9	11	2	28	3.8111	4	0.5–0.3	hom.
Cal-c−Y	1	34	34	37	25	2	132	2	7	34	21	6	70	32.7631	4	<0.01	het.
Canton−Y	1	34	30	50	73	10	197	36	33	38	51	3	181	14.1742	4	<0.01	het.
Dallas−Y	1	34	12	15	11	1	73	10	4	10	11	1	36	6.2514	4	0.2–0.1	hom.
Florida−Y	1	52	15	10	6	0	83	17	12	9	9	5	52	17.5478	4	<0.01	het.
Florida−Y	2	30	16	21	20	3	90	7	2	5	15	4	33	12.3565	4	0.02–0.01	het.
Formosa−Y	1a	51	27	11	5	0	94	10	13	15	5	0	43	16.3495	3	<0.01	het.
Formosa−Y	1b	18	17	15	7	0	57	3	4	12	8	0	27	9.6833	3	0.05–0.02	het.
Ft. Worth−Y	1+2	41	25	22	25	2	115	6	6	8	11	0	31	5.0210	4	0.3–0.2	hom.
Henderson−Y	1	48	26	24	39	8	145	8	7	12	36	6	69	19.3663	4	<0.01	het.
Kyoto−Y	1	9	10	27	53	22	121	3	6	12	32	4	57	5.0620	4	0.3–0.2	hom.
La Lajita−Y	1a	44	32	28	21	0	125	17	17	6	18	0	58	7.4812	3	0.1–0.05	hom.
Lausanne−Y	1	9	9	10	35	6	69	1	6	8	12	5	32	4.9275	4	0.3–0.2	hom.
Lubbock−Y	1	34	25	26	50	7	142	11	9	13	23	4	60	1.3597	4	0.9–0.8	hom.
Lubbock−Y	2	22	11	9	7	0	49	5	8	2	7	0	22	6.2693	3	0.1–0.05	hom.
Mexico 220.1−Y	1	16	13	13	12	2	56	5	6	7	20	3	41	3.1333	4	0.7–0.5	hom.
Seto−Y	1	37	26	36	57	7	163	14	10	36	31	3	94	8.8796	4	0.1–0.05	hom.
Swedish-b−Y	1	28	17	15	7	0	67	2	8	6	13	1	30	21.4848	4	<0.01	het.
Swedish-b−Y	2	19	7	7	16	0	49	2	2	8	23	0	35	15.9703	3	<0.01	het.
Tuscalosa−Y	1+2	66	29	40	43	1	179	11	13	27	39	6	96	29.2896	4	<0.01	het.
Urbana−Y	1	62	22	14	13	2	113	23	6	10	10	0	49	5.7000	4	0.3–0.2	hom.
Urbana−Y	2	31	14	21	4	0	70	7	3	11	4	0	25	5.2672	3	0.2–0.1	hom.
Uvalde−Y	1	57	21	12	4	0	94	11	13	12	14	1	51	29.3906	4	<0.01	het.
Woodbury−Y	1a	16	10	13	33	5	77	3	9	5	24	7	48	33.7220	4	<0.01	het.
Woodbury−Y	1b	19	11	11	13	0	54	1	4	2	15	1	23	30.3544	4	<0.01	het.

involving two or more series of the same cross, all series agreed in the matter of homogeneity or heterogeneity of wild type and $y^2\ v\ f$ intersexes.

It is not probable that the $y^2\ v\ f$ X chromosomes of the triploid stock varied with respect to intersex modifying genes, because of the long period

TABLE 5

Comparison of homogeneity tests of $y^2\ v\ f/+$ and $y^2\ v\ f/y^2\ v\ f$ intersexes in the two sets of experiments: (1) wild male×$y^2\ v\ f$ triploid and (2) wild male−Y×$y^2\ v\ f$ triploid.

MALE PARENT	HOMO-GENEITY	MALE PARENT	HOMO-GENEITY	MALE PARENT	HOMO-GENEITY
Amherst	hom.	Ft. Worth	hom.	Seto	hom.
Amherst−Y	hom.	Ft. Worth−Y	hom.	Seto−Y	hom.
Caddo Lake	het.	Henderson	het.	Swedish-b	hom.
Caddo Lake−Y	hom.	Henderson−Y	het.	Swedish-b−Y	het.
Cal-c	hom.	Kyoto	hom.	Tuscalosa	het.
Cal-c−Y	het.	Kyoto−Y	hom.	Tuscalosa−Y	het.
Canton	hom.	La Lajita	hom.	Urbana	hom.
Canton−Y	het.	La Lajita−Y	hom.	Urbana−Y	hom.
Dallas	het.	Lausanne	hom.	Uvalde	het.
Dallas−Y	hom.	Lausanne−Y	hom.	Uvalde−Y	het.
Florida	het.	Lubbock	hom.	Woodbury	hom.
Florida−Y	het.	Lubbock−Y	hom.	Woodbury−Y	het.
Formosa	het.	Mexico 220.1	hom.		
Formosa−Y	het.	Mexico 220.1−Y	hom.		

TABLE 6

Summary of homogeneity comparisons.

HOM.-HOM.	HET.-HET.	HOM.-HET.	HET.-HOM.
9	5	4	2
14		6	

of inbreeding of the triploid stock and also because the triploid stock originated as a single female.

As previously noted, the second set of experiments, summarized in table 4, was originally designed to test the possible presence of intersex modifying genes located in the Y chromosome, rather than in the X chromosome,

introduced by the wild male in the cross to $y^2\ v\ f$ triploids. Tables 5 and 6 compare the homogeneity tests of wild type and $y^2\ v\ f$ intersexes in the two sets of experiments. In nine cases the two kinds of intersexes were homogeneous in both sets of experiments. These nine stocks were Amherst, Ft. Worth, Kyoto, La Lajita, Lausanne, Lubbock, Mexico 220.1, Seto, and Urbana. In five cases, the wild type and $y^2\ v\ f$ intersexes were heterogeneous in both sets of experiments. The wild stocks producing heterogeneous intersexes in both table 3 and table 4 were Florida, Formosa, Henderson, Tuscalosa, and Uvalde (Uvalde is considered to produce heterogeneous intersexes, since three series out of four in table 3 gave heterogeneous intersexes; Uvalde−Y likewise produced hetereogeneous intersexes). Thus in 14 cases, experiments listed in tables 3 and 4 agreed.

In four cases, however, wild type and $y^2\ v\ f$ intersexes were homogeneous in the first set of experiments (table 3) and heterogeneous in the second set of experiments (table 4). The above four cases were Cal-c, Canton, Woodbury, and Swedish-b (Swedish-b is considered to produce homogeneous intersexes, since two series out of three in table 3 gave homogeneous intersexes).

In two cases, the two kinds of intersexes were heterogeneous in table 3 and then homogeneous in the second set of experiments in table 4. These stocks were Dallas and Caddo Lake.

While it is possible that these six cases which disagree in tables 3 and 4 may be explained by assuming that intersex modifying genes exist in the wild Y chromosome, another explanation is more plausible in view of the few genes known to be located in the Y chromosome. The sex types of intersexes depend of course upon the interaction between X chromosomes and autosomes. The wild type intersexes derived from corresponding crosses in both tables 3 and 4 possess a wild X chromosome from the same source and a $y^2\ v\ f$ X chromosome. However, the autosomes of the wild type intersexes of table 4 are more similar to those of their sib $y^2\ v\ f$ intersexes. This follows from the fact that to obtain wild males−Y (lacking the wild Y), the wild females of each stock were crossed to $y^2\ v\ f$ males derived from the triploid stock. The F_1 males, called wild males−Y, were then crossed to $y^2\ v\ f$ triploids to give progeny recorded in table 4. These males then possessed a Y chromosome and one of each autosome derived from the $y^2\ v\ f$ triploid stock. Hence in the wild type intersexes of table 4, the wild X chromosome genes were interacting with different autosomes from those with which the wild X chromosome of wild type intersexes of table 3 were interacting. This new interaction of the wild X chromosome genes and a different set of autosomes might change the homogeneity or heterogeneity of wild type and their $y^2\ v\ f$ sib intersexes. As table 6 shows, six such changes occurred.

DISCUSSION

Evidence has been offered that nine among the 20 wild stocks of *Drosophila melanogaster* investigated possessed X chromosomes with intersex modifying genes either of the same potency or recessive to those in the y^2 *v f* X chromosome of the triploid stock. Five of the remaining 11 wild stocks possessed X chromosomes which contained intersex modifying genes which altered the sex types of wild type intersexes from those of their y^2 *v f* sib intersexes regardless of the origin of the three sets of autosomes. Presumably these wild X chromosome modifying genes act by altering the time of the turning point between male and female development of the intersexes. DOBZHANSKY and BRIDGES (1928) have shown that Drosophila intersexes begin development as males; then a turning point occurs, and development is completed as females. The remaining six wild stocks of the 20 studied possessed X chromosome modifying genes which, it is believed, altered the sex types of wild type from those of their y^2 *v f* sib intersexes depending upon the degree to which the three sets of autosomes were derived from the y^2 *v f* triploid stock were present. This interaction of X and autosome intersex modifying genes is not surprising in view of DOBZHANSKY'S (1930) finding that intersex modifying genes existed in a particular chromosome III which were capable of shifting the sex type of the intersexes when present in only one dose.

The alternative hypothesis explaining lack of homogeneity or heterogeneity between corresponding wild type and y^2 *v f* intersexes in the two sets of experiments involving the preceding six wild stocks is that intersex modifying genes were present in the wild Y chromosome received by the y^2 *v f* intersexes in the first set of experiments. This hypothesis does not seem so plausible as the X-autosome interaction hypothesis because of the known scarcity of Y chromosome genes.

No correlation existed between geographical location of wild stocks and similarity of the wild X chromosome genes with the y^2 *v f* X chromosome. No such correlation was expected since the closest places of collection, Dallas and Ft. Worth, were some 32 miles apart.

The result of this study has been the demonstration of X chromosome intersex modifying genes of different potency from those in the y^2 *v f* X chromosome of the triploid stock in certainly five and probably six more (that is, 11) of the 20 wild stocks investigated. This phenotypically undetectable variation in geographically diverse wild populations is another example of the "small" mutations which contribute to evolutionary differentiation discussed by DOBZHANSKY (1937). A reason for such variation in action of sex genes in different wild populations is that natural selection has not had a chance to make the 2X 3A intersex condition as stable as the diploid sexes.

Although approximately half the different wild stocks showed a difference in the X chromosome intersex modifying genes when compared with the y^2 *v f* X chromosome, the individuals within a particular stock were remarkably uniform with respect to X chromosome intersex modifying genes. In only two cases was there any evidence of diversity of X chromosomes within a single stock.

SUMMARY

A study was made of intersex sex type modifying genes located in the X chromosomes of 20 different wild stocks collected from widely varying geographical regions. Males of each wild stock were crossed with y^2 *v f* triploids of a highly inbred line. Sex types of wild type and y^2 *v f* sib intersexes were compared. Eleven of the 20 wild stocks possessed X chromosomes with intersex modifying genes of different potency from those in the y^2 *v f* X chromosome. These wild X modifying genes could alter the sex types when present in only one dose.

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INHERITANCE OF FERTILITY IN THE LATERAL SPIKELETS OF BARLEY[1]

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INTRODUCTION

A STUDY was made of the inheritance of fertility in the lateral spikelets of the cultivated barley species in an attempt to explain some of the differences in fertility known to exist in certain crosses between species.

Four species of cultivated barley have been recognized by HARLAN (1918) on the basis of the fertility of the lateral spikelets, these being *Hordeum vulgare*, *H. intermedium*, *H. distichon*, and *H. deficiens*. *H. vulgare* and *H. intermedium* are so-called 6-rowed species with all spikelets at a rachis joint at least partially fertile, the latter species being different from the former in that the lateral spikelets are only partially fertile or, when fully fertile, without awns or hoods. *H. distichon* and *H. deficiens* are so-called 2-rowed species with no fertility of the lateral spikelets. The side spikelets consist of glumes, lemma, palea, rachilla, and usually the rudiments of the sexual organs in *H. distichon;* but they are reduced in *H. deficiens* to only the glumes and rachilla with rarely more than one flowering bract (lemma or palea) and never the rudiments of sexual organs.

It has been observed that some crosses give intermediums[2] which have fertile lateral spikelets, while others give only segregates with infertile lateral spikelets. There is a possibility that some factor or factors other than those for non-6-row versus 6-row (*Vv*) and for intermedium versus non-intermedium (*Ii*) are necessary to explain the difference in behavior between fertile and infertile intermediums.

[1] This material was taken from a thesis presented to the faculty of the Graduate School of the UNIVERSITY OF MINNESOTA in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The writer wishes to express his appreciation to DR. F. R. IMMER, Professor of Agronomy and Plant Genetics, UNIVERSITY OF MINNESOTA, for his many suggestions. This study was made under his direction. Credit is due DR. D. W. ROBERTSON, Agronomist, COLORADO EXPERIMENT STATION, for advice and guidance on many details involved in this investigation. The writer also wishes to express his appreciation to DR. H. K. HAYES, Professor of Agronomy and Plant Genetics, UNIVERSITY OF MINNESOTA, for his constructive criticism of the manuscript.

The data were all obtained at the COLORADO EXPERIMENT STATION at Fort Collins. Published with the approval of the Director of the Experiment Station as Scientific Series Paper No. 125.

[2] Both intermediums and intermediates may have partial lateral spikelet fertility. Intermediums (*VVII*) are distinguishable phenotypically by the rounded lemmas of the lateral florets which are never awn-pointed. Fertile intermediums are genetically *VV* with partial fertility of the lateral spikelets. Infertile intermediums (*VVII*) are designated as forms with rounded lemmas with less than two per cent of the lateral spikelets fertile. Ordinarily, there is no fertility of the lateral spikelets.

LITERATURE REVIEW

Previous research in barley genetics has been reviewed rather extensively by several investigators: GRIFFEE (1925), HAYES and GARBER (1927), ROBERTSON (1929), BUCKLEY (1930), KUCKUCK (1930a), and DAANE (1931). In addition, a bibliography on genetic factors has been compiled by ROBERTSON, WIEBE and IMMER (1941). Consequently the work reviewed here will be that which pertains particularly to the inheritance of fertility of the lateral spikelets.

A single-factor difference has been found between crosses of *Hordeum vulgare* and *H. distichon*, *H. vulgare* and *H. deficiens*, and *H. distichon* and *H. deficiens*. They have been considered as an allelic series by ENGLEDOW (1924) and by HOR (1924). The intermedium versus non-intermedium (*Ii*) factor pair was found by HARLAN and HAYES (1920) and by ROBERTSON (1929, 1933) to be independent of the non-6-row versus 6-row (*Vv*) factor pair.

Two distinct types of inheritance have been obtained from crossing varieties of *H. distichon* and *H. deficiens*, both 2-rowed species, with varieties of *H. vulgare*. These types of inheritance will be described in some detail.

Many workers (ROBERTSON, et al. 1941) have obtained three classes from crosses of *H. vulgare* and *H. distichon* varieties. An intermediate[3] condition was found to exist in the F_1 generation with the lateral spikelets awned (or hooded) and high in fertility. The F_2 segregation has been readily explained as a single-factor difference with a 1:2:1 ratio of 6-rowed intermediate, and 2-rowed forms.

That more than three classes for fertility and rows could be separated in F_2 generations of 6-row × 2-row crosses was pointed out by ENGLEDOW (1920), followed shortly afterwards by a complete genetical analysis by HARLAN and HAYES (1920). The latter gave their results for a cross between Manchuria, a 6-rowed barley, and Svanhals, a 2-rowed *H. distichon* variety. They obtained an F_1 with lateral spikelets slightly fruitful and with intermediate awns. The F_2 genotypes were classified into seven classes on the basis of their F_3 behavior. GRIFFEE (1925) found a similar condition in a Svanhals × Lion cross. ROBERTSON (1933) obtained similar results in a cross between Colsess and *H. deficiens nudideficiens*. The data have been explained on a two-factor basis—that is, intermediums versus non-intermediums (*Ii*) and non-6-row versus 6-row (*Vv*), with the intermedium factor pair (*Ii*) hypostatic to that for rows (*Vv*). The F_2 genotypes as determined by F_3 behavior are as follows:

[3] The term "intermediate" is used throughout this paper to designate the heterozygous condition of the non-6-row versus 6-row (*Vv*) factor pair. An intermediate may carry the genes *II*, *Ii*, *ii*, or any allele of this series.

CLASS	CLASSES WHICH SEGREGATE IN F_2	F_2 GENOTYPE	RATIO
1	6-row	*vvII* *vvIi* *vvii*	4
2	6-row, intermediate, and intermedium	*VvII*	2
3	6-row, intermediate, intermedium and 2-row	*VvIi*	4
4	6-row, intermediate, and 2-row	*Vvii*	2
5	Intermedium	*VVII*	1
6	Intermedium and 2-row	*VVIi*	2
7	2-row	*VVii*	1

That fertility of the lateral spikelets might be further influenced by minor modifying factors has been suggested by NEATBY (1929) and by HARLAN and HAYES (1920). GILLIS (1926) believes that a third factor pair (*Dd*) also is involved in fertility, being *D* in the intermediate forms and *d* in the *H. deficiens* types.

There is a possibility that some 2-rowed varieties of *H. distichon* really belong to the *H. intermedium* group genetically. This has long been suspected by HARLAN and MARTINI (1935) on the basis of extensive observations. They found occasional fertile lateral spikelets in 44 out of 408 supposedly normal 2-rowed varieties, or a total of 11 percent. Fertile lateral spikelets were especially plentiful in hybrid varieties and were much more common among dense-eared than among lax-eared barleys. These workers feel that different levels of fertility are correlated with a vigor of plant growth that may be significant. ENGLEDOW (1924) is also of the opinion that environment may influence fertility somewhat. In *H. intermedium haxtoni*, lateral floret fertility was influenced by time of planting, being much higher when planted early.

LINKAGE GROUPS IN BARLEY

Seven linkage groups have been established in barley. The literature on these groups has been reviewed by KUCKUCK (1930a, 1930b), DAANE (1931), ROBERTSON (1933, 1937, 1939), ROBERTSON, DEMING, and KOONCE (1932), and by ROBERTSON, WIEBE, and IMMER (1941). Since there are seven haploid chromosomes in cultivated barley, all linkage groups are thought to be found. As will be shown later, the factors concerned in this study are probably located in group IV. The factor pair for hoods versus awns (*Kk*) is located in this group. ROBERTSON (1933) found the factor pair for intermedium versus non-intermedium (*Ii*) to be linked with hoods versus awns (*Kk*) with 15.12 ± 0.065 percent recombination.

MATERIALS AND METHODS

This study on the inheritance of fertility of the lateral spikelets of barley

was made at the COLORADO EXPERIMENT STATION during the years 1931 to 1940, inclusive.

Symbols for genetic characters

The special fertility condition found in Mortoni involves the factor pair for non-6-row versus 6-row (*Vv*), but it must also be discussed in relation to the factor pair for intermedium versus non-intermedium (*Ii*). The genetic characters used in various crosses, together with their symbols, are listed below. The standard nomenclature suggested by ROBERTSON, WIEBE, and IMMER (1941) has been followed.

LINKAGE GROUP	CHARACTER PAIR	SYMBOLS
I	2-row versus non-2-row	*Vv*
	Green versus chlorina seedling color	*Ff*
II	Black versus white floral bracts	*Bb*
III	Covered versus naked caryopsis	*Nn*
IV	Hoods versus awns	*Kk*
	Intermedium versus non-intermedium (or 2-row)	*Ii*
V	Long versus short-haired rachillas	*Ss*
VI	Green versus xantha seedling color	*Xsxs*

Description of varieties used in experiments

The strains used in this experiment were as follows: Nudihaxtoni, Mortoni, Coast II, Smyrna I, Minnesota 84-7, Nilsson-Ehle No. 2, Nudideficiens, Nigrinudum, and Sublaxum. These varieties were made available by either the COLORADO EXPERIMENT STATION or the U. S. DEPARTMENT OF AGRICULTURE. Minnesota 84-7 came from the MINNESOTA AGRICULTURAL EXPERIMENT STATION.

H. intermedium nudihaxtoni (C.I. No. 2231) is an intermedium variety with complete fertility of the lateral spikelets. It is a naked barley with white kernels and floral bracts. The lemmas of the central spikelets are long-awned while those of the lateral spikelets are awnless.

H. intermedium mortoni (C.I. No. 2210) is a black, awned, covered variety with short-haired rachillas and purple plant color. It is a fertile intermedium with partial fertility of the lateral spikelets on practically every spike. (See fig. 1.) A count of the fertile and infertile lateral spikelets was made on the heads of seven parent plants used in crosses. From 32.2 to 51.2 percent of the lateral spikelets were fertile, the average being 40.7 percent.

The other varieties used in crosses generally were genetic testers. Except for Sublaxum, these strains have been described by previous workers (ROBERTSON 1929, 1933, 1937; ROBERTSON, DEMING, and KOONCE 1932). The factorial composition of the various parent lines is listed in table 1.

FIGURE 1 (left).—Heads of the Mortoni Parent. The partial fertility of the lateral spikelets is evident.

FIGURE 2 (right).—Classes in the F_2 generation of a Mortoni × Nigrinudum cross. The two heads on the left are infertile intermediums, the third head is a fertile intermedium, while the fourth is the type with inflated lateral spikelets classified as fertile.

Experimental methods

The experimental methods used were similar to those followed by ROBERTSON (1929). Particular attention has been given to the nature of fertility in the lateral spikelets. Three reciprocal crosses were made in each instance with the progeny carried to the F_3 generation. In the F_2 generation the plants were separated for the various character pairs being analyzed. Because of the difficulty in the separation of some of the classes, it was necessary in many cases to carry a random sample of the F_2 plants to the F_3 generation to determine the F_2 genotypes. A few heads were threshed from each F_2 plant, the seed of which was planted in individual 8-foot rows for the F_3 segregation.

The observed data were compared to calculated theoretical ratios and tested for goodness of fit by the use of χ^2 where two or more phenotypic

TABLE 1

List of the factor pairs found in the different parents.

VARIETY	SYMBOLS FOR CHARACTERS							
	Vv	*Ff*	*Bb*	*Nn*	*Kk*	*Ii*	*Ss*	*Xsxs*
Coast II*	*vv*	*FF*	*bb*	*NN*	*kk*	*II*	*ss*	*XsXs*
Minnesota 84-7	*vv*	*ff*	*bb*	*NN*	*kk*	—	*SS*	*XsXs*
Nilsson-Ehle No. 2†	*VV*	*FF*	*bb*	—	*kk*	*ii*	—	*XsXs*
Smyrna I	*VV*	*FF*	*bb*	—	*kk*	*ii*	—	*XsXs*
Nudihaxtoni	*vv*	*FF*	*bb*	*nn*	*kk*	*II*	*SS*	*XsXs*
Mortoni	*VV*	*FF*	*BB*	*NN*	*kk*	—	*ss*	*XsXs*
Sublaxum	*VV*	*FF*	*bb*	*nn*	*KK*	*ii*	—	*XsXs*
Nudideficiens	*VV*	*FF*	*bb*	*nn*	*kk*	*ii*	*SS*	*XsXs*
Nigrinudum	*VV*	*FF*	*BB*	*nn*	*kk*	*II*	*ss*	*XsXs*

* Coast II is heterozygous for green versus white seedlings (*Ac2 ac2*).

† Nilsson-Ehle No. 2 is heterozygous for albino seedlings (*A2a2*). These factor pairs segregated for 3:1 ratio in each case. Since they were not used in linkage relations, they will not be discussed further.

classes were involved. The interpretation of the significance of χ^2 values was the same as that used by FISHER (1934). The formula applied for the computation of χ^2 for a 3:1 ratio was as follows: $\chi^2 = (A - 3a)^2/3N$ where A = the observed number in the dominant class, a = the observed number in the recessive class, and N = the total number of individuals. The probability value for one degree of freedom was determined by reference to a normal probability integral table.

The linkage determinations from F_2 data were made by the use of the product formula. The recombination percentages, as well as their standard errors, were determined from tables prepared by IMMER (1930). The F_2 and F_3 data were combined for the determination of the recombination value by the method suggested by IMMER (1934) as modified by ROBERTSON and COLEMAN (1940) for particular cases.

EXPERIMENTAL RESULTS

The experimental results will be presented in three parts—namely, (1) determination of the constitution of the parent material for non-6-row versus 6-row (*Vv*) and for intermedium versus non-intermedium (*Ii*), (2) inheritance of the fertile intermedium condition in Mortoni, and (3) the interrelationships of the factors concerned with lateral spikelet fertility with other factor pairs located in known linkage groups.

Row and intermedium constitution of parental material

It was first necessary to determine the genetic constitution of the parent material for the non-6-row versus 6-row (*Vv*) and intermedium versus non-

intermedium factor pairs. The various lines studied were crossed on others of known constitution as determined by ROBERTSON (1929, 1937). These testers were: Nigrinudum (*VVII*), Nudideficiens (*VVii*), and Coast II (*vvII*). Nigrinudum is genotypically an infertile intermedium. From crosses with these testers, the factorial compositions of the parental lines were established as follows: Nudihaxtoni, *vvII;* Sublaxum, *VVii;* and Mortoni, *VV*. It was apparent that Mortoni, morphologically a *H. intermedium* variety, has the same composition for the 2-row (*VV*) condition as do some *H. distichon* varieties. It differs from the latter in that it is homozygous for partial fertility of the lateral spikelets. The intermedium versus non-intermedium (*Ii*) condition in Mortoni may not be the same as that in the other lines mentioned.

When the various known factorial constitutions for the row (*Vv*) and intermedium (*Ii*) factor pairs are summarized in the light of their F_1 phenotypic behavior, several facts are immediately apparent. Partial fertility of the lateral spikelets in the F_1 is due to the homozygous dominant intermedium, *II*, factorial condition in the presence of a heterozygous condition for rows, *Vv*. Non-fertility of the lateral spikelets in the F_1 is due to the presence of the heterozygous condition of the intermedium versus non-intermedium (*Ii*) factor pair in the presence of either *VV* or *Vv*. This is indicated in table 2.

TABLE 2

Fertility of the lateral spikelets in the F_1 generation for various genotypes for rows and intermediums in various crosses.

CROSS	FERTILITY CONDITION IN F_1 PLANTS	
	GENOTVPE	PHENOTYPE
Nudihaxtoni (*vvII*)×Nigrinudum (*VVII*)	*VvII*	Partial fertility
Coast II (*vvII*)×Nigrinudum (*VVII*)	*VvII*	Partial fertility
Coast II (*vvII*)×Nudideficiens (*VVii*)	*VvIi*	No fertility
Coast II (*vvII*)×Sublaxum (*VVii*)	*VvIi*	No fertility
Sublaxum (*VVii*)×Nigrinudum (*VVII*)	*VVIi*	No fertility

It is obvious that this fertile intermedium condition involves a heterozygous condition for the non-6-row versus 6-row (*Vv*) factor pair and the presence of the homozygous dominant intermedium (*II*) factor. The inheritance of the partially fertile intermedium as a homozygous condition is still to be considered.

The fertile intermedium condition in Mortoni

The factor pair for non-6-row versus 6-row (*Vv*) was studied in a cross between Mortoni and Nudihaxtoni. The F_1 plants were fertile intermedi-

ums. The lateral spikelets were 75 to 90 percent fertile, but with no awns or tip-awns on any of the lemmas. Three classes were separable in the F_2 generation—namely, 2-row, partially fertile intermediums, and 6-row. The observed F_2 data were calculated on the basis of a 1:2:1 ratio on the hypothesis that the observed classes could be explained by the non-6-row versus 6-row (*Vv*) factor pair. The data appear in table 3. The observed data afforded a poor fit to the calculated 1:2:1 ratio for non-6-row versus 6-row (*Vv*).

TABLE 3

Segregation in the F_2 generation for 2-row versus intermediates versus 6-row (Vv) in a cross between Mortoni and Nudihaxtoni.

ITEM	NUMBER OF PLANTS WITH CHARACTERS INDICATED			
	2-ROW	INTERMEDIATES	6-ROW	TOTAL
Observed counts	117	390	195	702
Calculated 1:2:1 ratio	175.50	351.00	175.50	702

$\chi^2 = 26.000$ $P = 0.000002$

The observed F_2 data were then calculated on a two-factor basis for the non-6-row versus 6-row (*Vv*) and for the intermedium versus non-intermedium (*Ii*) factor pairs. The data indicate two levels of fertility for intermedium—namely, partially fertile intermedium like the Mortoni parent and infertile intermedium as found in many 2-row sorts. The observed F_2 data, calculated on a two-factor basis with the intermedium condition hypostatic to the 6-row (*vv*) factor, are given in table 4. The observed data afforded a good fit to the calculated 3:9:4 ratio for a two-factor difference.

TABLE 4

Segregation in the F_2 generation for non-6-row versus 6-row and for infertile versus fertile intermedium in a Mortoni×Nudihaxtoni cross.

ITEM	NUMBER OF PLANTS WITH CHARACTERS INDICATED			
	INTERMEDIUM		6-ROW	TOTAL
	INFERTILE	FERTILE		
Observed counts	117	390	195	702
Calculated 3:9:4 ratio	131.62	394.88	175.50	702

$\chi^2 = 3.8510$ $P = 0.1484$

The F_2 data for the fertility of the lateral spikelets can be explained tentatively on the basis of the factor pair for non-6-row versus 6-row (*Vv*) and on the assumption of a single-factor difference for infertile versus fertile intermedium. It appears that an allelic series exists for intermedium. The

intermedium condition, reported by ROBERTSON (1929), has non-fertile lateral spikelets and has been designated as *II*, while the non-intermedium allele has been designated as *ii*. Varieties with either factor pair in the homozygous condition are morphologically 2-rowed sorts in that they have only one fertile spikelet per rachis joint. Another allele found in the Mortoni parent, which breeds true for fertility on many lateral spikelets, will be designated as fertile intermedium (I^hI^h). This allele appears to be similar to the *BB* factorial condition found in the homozygous intermedium, Haxtoni (*aaBB*), reported by HARLAN and HAYES (1920). The infertile intermedium condition is dominant over partial fertility. All intermediums are genotypically *VV*. The cross, Mortoni (VVI^hI^h) × Nudihaxtoni (*vvII*), would be expected to segregate as follows in the F_2 and F_3 generations:

GENOTYPE	FREQUENCY	F_2 PHENOTYPES	RATIO	BEHAVIOR IN F_3 GENERATION
VVII	1	Infertile intermedium	3	Infertile intermedium
$VVII^h$	2	Infertile intermedium		Segregates 3:1 for infertile vs. fertility
VvII	2	Fertile intermediate	9	Segregates 1:2:1 for infertile, partial fertility and 6-row
$VvII^h$	4	Fertile intermediate		Segregates 3:9:4 same as F_2
VVI^hI^h	1	Fertile intermedium		Fertile intermedium
VvI^hI^h	2	Fertile intermediate		Segregates 3:1 for fertile intermedium vs. 6-row
vvII	1	6-row	4	6-row full fertility
$vvII^h$	2	6-row		6-row full fertility
vvI^hI^h	1	6-row		6-row full fertility

The F_2 plants were grown in the F_3 generation to determine their genotypes. The *VvII* and $VvII^h$ classes, being very difficult to separate in the field, were grouped together. As predicted from the F_2 data, the homozygous partially fertile intermedium (VVI^hI^h) plants had been grouped with the intermediate class for rows (*Vv*). The observed data presented in table 5 afforded a good fit to the calculated 1:2:1:6:2:4 ratio for independent inheritance of the non-6-row versus 6-row (*Vv*) and infertile versus fertile intermedium (II^h) factor pairs. An allelic series for the intermedium condition is indicated.

To verify the assumption that an allelic series for intermedium is involved, the three intermedium conditions, *II*, I^hI^h, and *ii*, were tested in crosses which involved all possible combinations of these factors.

The inheritance of non-intermedium (*ii*) versus fertile intermedium (I^hI^h) was studied in crosses between Mortoni (VVI^hI^h) and other varieties which were *VVii* in constitution. The F_1 plants were non-intermedium with no fertility of the lateral spikelets—that is, the non-intermedium condition was dominant. The F_2 plants were classified as non-intermedium (*VVii* and

TABLE 5

Observed and calculated F_2 genotypes for non-6-row versus 6-row and infertile versus fertile intermedium as determined by the F_2 segregation in a Mortoni×Nudihaxtoni cross.

ITEM	NUMBER OF INDICATED GENOTYPE						
	VVII	*VVII^h*	*VVI^{h}I^h*	*VvII & VvII^h*	*VvI^{h}I^h*	6-ROW*	TOTAL
Obsvd. count	43	79	36	289	82	173	702
Calc. ratio†	43.88	87.75	43.87	263.25	87.75	175.50	702

* 6-row includes *vvII*, *vvII^h*, and *vvI^{h}I^h*.

† Compared with a calculated 1:2:1:6:2:4 ratio. $\chi^2=5.2331$. P=0.3903.

VVII^h) and fertile intermedium (*VVI^{h}I^h*). On account of the difficulty in separation of the F_2 classes, seed from the individual F_2 plants was grown in the F_3 generation to obtain the F_2 genotypes, the data for which are given in table 6. The data indicate that non-intermedium (*ii*) versus fertile intermedium (*I^{h}I^h*) is the result of the segregation of a simple Mendelian factor pair.

TABLE 6

Observed and calculated F_2 genotypes for non-intermedium (or 2-row) versus fertile intermedium as determined by the F_2 segregation in crosses between Mortoni and other varieties.

CROSS	NUMBER OF INDICATED GENOTYPE*					
	NON-INTERMEDIUM (*ii*)	HETEROZYGOTES (*I^{h}i*)	FERTILE INTERMEDIUM (*I^{h}I^h*)	TOTAL	χ^2	P
Nudideficiens×Mortoni	266	475	227	968	3.4774	0.1812
Nilsson-Ehle No. 2×Mortoni	126	256	118	500	0.5440	V. large
Sublaxum×Mortoni	201	429	197	827	1.2007	0.5586
Mortoni×Smyrna I	24	35	23	82	1.7805	0.4203
Minnesota 84-7×Mortoni	129	225	101	455	3.5011	0.1791

* Compared with a calculated 1:2:1 ratio.

A Sublaxum (*VVii*)×Nigrinudum (*VVII*) cross was used to study the inheritance of infertile intermedium versus non-intermedium (*Ii*). The F_2 data, presented in table 7, indicate a single-factor difference, although the fit of the total observed data to the calculated 3:1 ratio was not good. The discrepancy appears to be due to the II-32-149 cross in which the non-intermedium forms were consistently high. This is possibly due to the difficulty of separation of the two classes in the F_2 generation.

Nigrinudum (*VVII*) was crossed with Mortoni (*VVI^{h}I^h*), the F_3 data for which are presented in table 8. The F_2 data were classified so as to include a few plants with inflated but non-fertile lateral spikelets in the fertile inter-

medium class. (See fig. 2.) This appeared to be justified, since occasional plants of the Mortoni parent fail to show any fertility of the lateral spikelets. The observed data afford a good fit to the calculated 1:2:1 ratio for a monohybrid segregation.

TABLE 7

Segregation in the F_2 generation for intermedium and non-intermedium in a Sublaxum×Nigrinudum cross.

CROSS NO.	OBSERVED NUMBERS*			χ^2	P
	INTER-MEDIUM	NON-INTER-MEDIUM	TOTAL		
II-32- 55	262	90	352	0.0606	0.8026
-147	195	81	276	2.7826	0.0949
-148	230	89	319	1.4305	0.2301
-149	239	108	347	6.9404	0.0085
-150	223	87	310	1.5527	0.2113
Totals	1149	455	1604	9.6958	0.0019

* Compared with a calculated 3:1 ratio.

Since a monohybrid segregation was obtained in crosses with the infertile intermedium (*II*), non-intermedium (*ii*), and fertile intermedium (I^hI^h) factors taken in all possible combinations, a multiple allelic series is definitely indicated.

TABLE 8

Observed and calculated F_2 genotypes for infertile versus fertile intermedium as determined by the F_3 segregation in a Mortoni×Nigrinudum cross.

ITEM	NUMBER OF INDICATED GENOTYPE			TOTAL
	INFERTILE INTERMEDIUM (*II*)	HETERO-ZYGOTES (II^h)	FERTILE INTERMEDIUM (I^hI^h)	
Observed count	101	202	81	384
Calc. 1:2:1 ratio	96	192	96	384

$\chi^2=3.1250$ $P=0.2122$

The data for one Coast II×Mortoni cross indicate that some Coast II plants carry the fertile intermedium allele (I^hI^h). The F_1 plants in this particular case had fully fertile lateral spikelets with tip awns less than one-half the length of the awns on the central spikelets. The F_2 plants were classified as fertile intermediums, intermediates, and 6-row. All intermediums showed some fertility of the lateral spikelets. The F_2 plants grown in

the F_3 generation showed all intermedium genotypes to have fertile lateral spikelets. The infertile intermedium form was not recovered.

Since the intermedium allelic series is hypostatic to the 6-row (*vv*) condition, it is necessary to determine the intermedium constitution of 6-rowed varieties used in crosses where fertility is being studied genetically. The value of known testers is obvious. An infertile intermedium 2-rowed variety such as Nigrinudum (*VVII*) could be used to test the constitution of unknown 6-rowed and 2-rowed varieties for the intermedium allelic series as follows:

(a) Unknown 6-row (*vv*—) × *VVII* gives non-fertile lateral spikelets in F_1 when the 6-rowed variety is *vvii*. Non-intermedium forms are recovered in F_2 and F_3

(b) Unknown 6-row (*vv*—) × *VVII* gives partial lateral spikelet fertility in the F_1 when the 6-rowed variety is either *vvII* or vvI^hI^h. In case it is *vvII* the F_2 will segregate into three classes in a 1:2:1 ratio—namely, infertile intermediums, fertile intermediates, and 6-row. This segregation may be verified in the F_3 generation. When the unknown 6-rowed variety is vvI^hI^h, the F_2 will give infertile intermediums, fertile intermediums and fertile intermediates (indistinguishable), and 6-row. The F_3 segregation will give infertile intermediums and fertile intermediums among the seven phenotypic classes.

(c) The *H. distichon* and H. *deficiens* varieties—that is, morphological 2-rowed varieties, may be crossed with Nigrinudum to determine their constitution for *II* or *ii*.

Factor interrelationships

Mortoni, classified as *H. intermedium* by HARLAN (1918), appears to be genetically VVI^hI^h. The interrelationships of the partial lateral spikelet fertility factor with others in known linkage groups were studied with the view that the fertility condition was due to another allele, (I^hI^h). Two-row non-intermedium (*VVii*) testers were used in the study of interrelationships with the non-intermedium versus fertile intermedium (I^hi) factor pair.

Characters inherited independently

The various characters inherited independently of the non-intermedium versus fertile intermedium (I^hi) factor pair in Mortoni crosses are given in table 9. The F_1 plants in all cases were characterized by infertile lateral spikelets. The F_2 plants were classified for non-intermediums, fertile intermediums, and the other factor pairs studied in particular crosses. Because of the difficulty in classification, the selfed F_2 plants were grown in the F_3 generation to determine the F_2 genotypes.

The segregations in the F_3 generation indicate a good agreement between

TABLE 9

Observed F_2 genotypes for non-intermedium versus fertile intermedium and factors inherited independently as determined by F_2 segregations in crosses of Mortoni with other varieties.

MORTONI CROSSED WITH:	GENOTYPE STUDIED	LINKAGE GROUP	GENOTYPES* AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb	TOTAL	χ^2	P
Minn. 84-7	I^hiFf	I	41	62	26	63	106	56	25	55	21	455	9.8431	0.2772
Nudideficiens	I^hiBb	II	62	127	77	119	232	124	45	117	65	968	9.7934	0.2810
N. Ehle No. 2	I^hiBb	II	31	65	30	55	130	71	26	60	32	500	3.4080	0.9028
Nudideficiens	I^hiNn	III	58	138	70	116	245	114	61	108	58	968	6.1363	0.6322
Nudideficiens	I^hiSs	V	47	60	24	60	135	72	22	59	33	512	14.4610	0.0713
Smyrna I	I^hiXsXs	VI	9	15	—	9	26	—	7	16	—	82	2.8802	0.7179

* Compared with calculated 1:2:1:2: 4:2:1:2:1 ratio in all cases except Mortoni×Smyrna I cross which was compared with calculated 1:2:2:4:1:2 ratio.

the observed data and the calculated ratios for independent inheritance. It appears that the factor pair for non-intermedium versus fertile intermedium (I^hi) is inherited independently of factor pairs as follows: Green versus chlorina (Ff) located in linkage group I, black versus white (Bb) floral bracts known to be in group II, covered versus naked seeds (Nn) located in group III, long versus short-haired rachillas (Ss) previously placed in group V, and green versus xantha seedling color ($XsXs$) known to be in group VI. The non-intermedium versus fertile intermedium (I^hi) factor pair was not tested with factors located in group VII.

Linkage of characters

The interrelationship of the character pairs for non-intermedium versus fertile intermedium (I^hi) and hoods versus awns (Kk) was tested in a cross between Sublaxum and Mortoni. The factor pair for hoods versus awns (Kk) is known to be located in group IV. The F_1 plants were 2-rowed, hooded, and with non-fertile lateral spikelets. The observed F_2 data for these characters are given in table 10.

The observed data afford a poor fit to the calculated 9:3:3:1 ratio for independent inheritance. It is noted that the non-intermedium awned and fertile intermedium hooded plants are fewer than expected for independent inheritance. These are recombination classes, since the Sublaxum parent was non-intermedium hooded and the Mortoni parent fertile intermedium awned. Linkage in the coupling phase is indicated. The recombination percentage, calculated by the product method, was found to be 13.12 ± 1.27. The fit of the observed data to the calculated segregation for 13.12 percent recombination is very good. A linkage of the factor pairs for non-intermedium versus fertile intermedium (I^hi) and hoods versus awns (Kk) is indicated. These data verify the hypothesis that an allelic series exists for intermedium—that is, I, I^h, and i.

TABLE 10

Segregation in the F_2 generation for non-intermedium versus fertile intermedium and hoods versus awns in a Sublaxum×Mortoni cross.

ITEM	NUMBER OF PLANTS WITH CHARACTERS INDICATED					χ^2	P
	NON-INTERMEDIUM		FERTILE INTERMEDIUM		TOTAL		
	HOODS	AWNS	HOODS	AWNS			
Observed count	588	49	50	144	831		
Calc. 9:3:3:1 segregation	467.44	155.81	155.81	51.94	831	339.3392	V. small
Calc. 13.12 percent recombination	572.31	50.94	50.94	156.81	831	1.5678	0.6713

The data for the F_2 phenotypes and F_2 genotypes from the 9:3:3 classes in F_2 were combined for the calculation of linkage by use of the formulae suggested by IMMER (1934). The formula for the calculation of linkage intensities for F_2 genotypes as determined from the segregation for the 9:3:3 F_2 phenotypic classes is as follows:[4,5]

$$\frac{2e+f+g+k+m}{p}-\frac{f+g+2j+k+2l+m}{1-p}+\frac{2(j+k+l+m)p}{1-p^2}$$
$$-\frac{2(h+i)(1-2p)}{1-2p+2p^2}-\frac{2(e+f+g+h+i)p}{2+p^2}=0.$$

The information in F_3 for n plants is:

$$\frac{4n(6-10p+5p^2-4p^3+20p^4+2p^5+2p^6)}{p(1-p)(1+p)^2(2+p^2)^2(1-2p+2p^2)}.$$

The recombination value for the combined F_2 phenotypes and genotypes was found to be 14.32 ± 0.61 percent. The observed data for the F_2 genotypes as determined from the F_3 segregation are given in table 11.

The F_3 data gave a good fit to the calculated ratio with 14.32 percent recombination, which indicates that the non-intermedium versus fertile intermedium (I^hi) and hoods versus awns (Kk) factor pairs are in linkage group IV.

[4] Modification for 9:3:3 class worked out by O. H. COLEMAN, COLORADO EXPERIMENT STATION.

[5] Symbols used to designate the observed frequency of occurrence of individuals in various genotypes:

	AA	Aa	aa
BB	e	f	l
Bb	g	h i	m
bb	j	k	n

TABLE 11

Fit of F_2 genotypes in a Mortoni×Sublaxum cross to a calculated ratio with 14.32 percent recombination.

ITEM	NUMBER OF PLANTS OF INDICATED GENOTYPE								TOTAL
	iiKK	*iiKk*	*iikk*	I^hiKK	I^hiKk	I^hikk	I^hI^hKK	I^hI^hKk	
Observed count	153	44	2	57	331	43	6	46	682
Calc. 14.32 percent recombination	153.30	51.24	4.28	51.25	315.16	51.24	4.28	51.25	682

$\chi^2=6.2335$ $n^1=8$ $P=0.5139$

DISCUSSION OF RESULTS

The results of this study indicate that partially fertile intermediates obtained in the F_1 generation from 2-row and 6-row barley crosses are heterozygous for the non-6-row versus 6-row (*Vv*) factor pair and homozygous dominant for infertile intermedium (*II*). The complete lack of lateral spikelet fertility found in F_1 plants apparently results when the non-6-row condition (*VV* or *Vv*) is present with the infertile intermedium versus non-intermedium factor pair in the heterozygous condition (*Ii*).

Some varieties of barley classified as *Hordeum intermedium* have partial fertility of the lateral spikelets that breeds true. This was found to be true of Mortoni (C.I. 2210). The results of this investigation indicate Mortoni to be a fertile intermedium (VVI^hI^h) barley with 10 to 50 percent of the lateral spikelets generally fertile. The data obtained indicate that partial lateral spikelet fertility in Mortoni is due to another allele of the infertile intermedium versus non-intermedium (*Ii*) factor pair which has been designated as fertile intermedium (I^hI^h). The allelic series for intermedium has been established as *I*, I^h, and *i*. These three intermedium conditions (*II*, I^hI^h, and *ii*) were tested in crosses of parents that carry these factors in all possible combinations. A monohybrid segregation was obtained in each case, a condition necessary for multiple alleles. Non-fertility appeared to be dominant over fertility in every instance. The intermedium allelic series, together with the expected classes in crosses between 6-rowed and 2-rowed barley varieties, in the F_2 generation, may be summarized as follows:

While a single factor appeared to explain the fertility condition found in Mortoni, it was also observed that environmental conditions influence fertility to some extent. In crosses of Mortoni with some 6-rowed varieties, it was also evident that minor modifying factors possibly influence fertility of the lateral spikelets. Fertility of the lateral spikelets was more evident in space-planted F_2 rows than in drilled F_3 rows. Some plants genetically fertile failed to develop fertility in thick stands in the drilled rows. In studies of the intermedium series, it is recommended that the selfed F_2

CLASS	PHENOTYPIC CLASSES	F_2 GENOTYPES					
		Ii		II^h		I^hi	
		(From F_3 Segregation)					
1.	6-rowed*	*vvII* *vvIi* *vvii*	4	*vvII* *vvI^{h}I* *vvI^{h}I^h*	4	*vvI^{h}I^h* *vvI^{h}i* *vvii*	4
2.	6-rowed, intermediates, intermediums						
	(a) Fertile intermediums	—		*VvI^{h}I^h*		*VvI^{h}I^h*	2‡
	(b) Infertile intermediums	*VvII*	2	*VvII*	2	—	
	(c) Fertile and infertile intermediums	—		*VvII^h*	4	—	
3.	6-rowed, intermediates, intermediums, 2-rowed†						
	(a) Fertile intermediums	—		—		*VvI^{h}i*	4‡
	(b) Infertile intermediums	*VvIi*	4	—		—	
4.	6-rowed, intermediates, 2-rowed	*Vvii*	2	—		*Vvii*	2‡
5.	Intermediums						
	(a) Fertile intermediums	—		*VVI^{h}I^h*	1	*VVI^{h}I^h*	1
	(b) Infertile intermediums	*VVII*	1	*VVII*	1	—	
	(c) Fertile and infertile intermediums	—		*VVI^{h}I*	2	—	
6.	Intermediums and 2-rowed						
	(a) Fertile intermediums	—		—		*VVI^{h}i*	2
	(b) Infertile intermediums	*VVIi*	2	—		—	
7.	2-rowed	*VVii*	1	—		*VVii*	1

* The 6-row (*vv*) factor is epistatic to the intermedium alleles (*I*, I^h, and *i*). These genotypes are never observed.

† Two-rowed is considered here as *VVii*.

‡ Segregation not actually observed.

plants of the fertile intermedium class be space-planted in the F_3 generation to permit the most favorable environmental conditions for the development of fertility of the lateral spikelets. This is particularly desirable for crosses like Mortoni × Nigrinudum that involve segregation of the infertile versus fertile intermedium (II^h) factor pair. In this cross it was difficult to distinguish between the homozygous fertile intermedium (I^hI^h) rows and those heterozygous for the infertile and fertile condition (II^h).

The non-intermedium versus fertile intermedium (I^hi) factor pair appeared to be linked with that for hoods versus awns (*Kk*) located in group IV with a recombination value of 14.32 ± 0.61 percent as determined from the combined F_2 phenotypes and genotypes. Such a linkage would be expected on the hypothesis of an allelic series for intermedium.

From the evidence available, it appears that there has been a change in the use of the term intermedium from its original form. HARLAN and HAYES

(1920) indicated that their homozygous intermedium barley, Haxtoni, designated by them as *aaBB*, had lateral spikelet fertility that averaged from 17.8 to 54.6 percent in different F_3 progeny lines. It seems reasonable to conclude that this is the intermedium described by students of barley classification. The intermedium factor (I^hI^h) defined in this paper appears to be similar to the *BB* factor reported by HARLAN and HAYES (1920). The fertile intermedium condition in Mortoni (VVI^hI^h) is probably identical with that described for the homozygous intermedium, Haxtoni. Homozygous fertile segregates were obtained in the F_3 generation for various crosses of Mortoni with both 2-rowed and 6-rowed varieties. The establishment of an allelic series for intermedium (I, I^h, and i) removes the confusion that has prevailed in the interpretation of the various intermedium conditions.

SUMMARY

A study was made to determine the inheritance of fertility in the lateral spikelets of the Mortoni variety of barley classified as *H. intermedium*.

The fertile intermedium condition found in the F_1 generation in crosses between some 2-row and 6-row barleys is due to the genetic constitution *VvII*.

Homozygous partial lateral spikelet fertility found in the Mortoni variety appeared to be due to a fertility allele of the infertile intermedium versus non-intermedium (*Ii*) factor pair. The allelic series for intermedium appears to be I, I^h, and i, with infertility dominant over fertility.

The non-intermedium versus fertile intermedium (I^hi) factor pair, from the combined F_2 and F_3 data, was found to be linked with that for hoods versus awns (*Kk*) with 14.32 ± 0.61 percent recombination. Hoods versus awns (*Kk*) is known to be located in group IV. The non-intermedium versus fertile intermedium (I^hi) factor pair was found to be inherited independently of factors known to be located in groups I, II, III, V, and VI. It was not tested with factors in group VII.

A method of testing barley varieties for intermedium constitution is suggested.

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GENETICS OF NATURAL POPULATIONS. VI. MICROGEOGRAPHIC RACES IN LINANTHUS PARRYAE

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INTRODUCTION

THE differentiation of a species into geographic races is a familiar phenomenon. Such races, usually referred to as subspecies by the systematist, consist of populations which exhibit a characteristic facies throughout a given geographic area. Although the populations may themselves be disjunct, the area which they occupy is essentially continuous. Such races are usually not homogeneous, unless greatly restricted, but present minor variants of at least two sorts. Some reflect an adaptive response of the species to the local environment; they tend to recur wherever the proper environment is found within the distribution area of the species; they are known as ecotypes (TURESSON 1922). Other local variants display no apparent relation to a particular environment; for such variants the noncommittal term "microgeographic races" has been proposed (DOBZHANSKY 1937). Microgeographic races are of interest both to genetics and to systematics because they may represent one of the first steps in the process of spatial diversification of species. Investigation of this phenomenon may therefore shed light on the structure and the dynamics of natural populations. The present paper describes the results of an exploratory study of microgeographic races in *Linanthus Parryae*. The races studied differ in the relative frequencies of white and blue flowered plants.

The writers wish to express their appreciation to MR. HAROLD LINT, MR. DONALD RAPPOPORT, and MR. SEYMOUR SINGER for their help in collecting the data and to PROFESSOR SEWALL WRIGHT for advice regarding statistical procedure.

MATERIAL

Linanthus Parryae (Gray) Greene, a representative of the Polemoniaceae, is a diminutive annual of the Mojave Desert, California. Its stem is commonly the diameter of a pencil lead and sometimes as much as 10 cm tall, but usually less and often scarcely 1 cm.; it may become intricately branched. The flowers are large relative to the vegetative parts of the plant. The corolla is commonly 1–1.5 cm. long, with a spread of petals of as much as 2 cm. The number of flowers may vary from one to as many as 243 per plant, the usual number being 5–10. Several flowers may be open at the

same time on a given plant, and most of the plants within a given population flower simultaneously. The period of flowering varies usually from two to three weeks and occurs in April or May. The plant is inconspicuous before flowering, and several hundred may lie underfoot but be scarcely observed against the desert gravel. In flower and en masse they are conspicuous and showy, as attested by the name "desert snow."

Two types of flower color occur in *Linanthus Parryae:* white and blue. In the former, the prevailing white is relieved by a band of purple in the throat. The blue is constant neither in shade nor in intensity, but varies from violet to a more reddish purple. Nevertheless, the difference between white and blue is always discernible to a practiced eye whether the corolla is living or withered. In one instance a blue flowered plant was found on which the two flowers upon one branch were white.

DISTRIBUTION AND HABITAT

Linanthus Parryae occurs around the southern and western margins of the Mojave Desert. The area described in this paper, the population of which has been studied in detail (maps in fig. 1 and 2), lies to the north of San Gabriel and San Bernardino Mountains in Los Angeles and San Bernardino Counties, California. This area is a gently rolling piedmont, sloping northward from an elevation of approximately 3,000 feet to approximately 2500. It is terminated on the south by the mountain ranges mentioned above, and on the north by the more alkaline valley floor. The piedmont is homogeneous but not uniform in aspect, structure, and soils. It is trisected from south to north by the stream bed of the Mojave river (at stations 192 to 195 and at Victorville; see fig. 2) and by Big Rock Creek (northwest of Llano) and Little Rock Creek (east of Palmdale, fig. 1) which rise in the San Bernardino and San Gabriel Ranges. All are intermittent, at least in the lower part of the slope. Their courses, like those of many desert streams, are inconstant. In addition to these streams a wet year produces smaller ephemeral ones which may flood the piedmont in their courses, depositing coarse gravel or rock, or silt, or carving a shallow and erratic channel.

Exclusive of the stream beds, in the detritus of which *Linanthus Parryae* does not occur, the principal soils of the area are as follows: Adelanto sand, Adelanto loamy sand, Adelanto sandy loam, Hesperia loamy sand, and Hesperia loamy fine sand. Only the areas in the vicinity of Palmdale and Victorville have been soil surveyed, however. Because the short hair-like roots of Linanthus would probably be but little affected by the nature of the subsoil during their short course of life, the actual variation in soils with reference to this species is less than appears from the above list, and the soils of the whole area can be taken as relatively uniform. They are similar

in their alluvial origin from granitic rocks. We are indebted to PROFESSOR M. R. HUBERTY for the above interpretation.

The vegetation of the piedmont is also homogeneous. It falls chiefly within the Larrea-Franseria association, the characteristic cover of the Mojave and Colorado deserts. The most widespread perennial is *Larrea divaricata*. Associated with it are such species as *Eriogonum polifolium*, *Ephedra californica*, *Dalea Schottii*, *Haplopappus linearifolius*, *Hymenoclea Salsola*, and in some areas the grasses *Oryzopsis hymenoides*, *Stipa speciosa*, and *Hilaria rigida*. *Yucca brevifolia* is scattered throughout most of the area. *Juniperus californica* enters from the mountain slopes at the southern margin, and Atriplex communities are usually found in the valley floor at the foot of the piedmont. The spacing of these shrubs is wide, and it is doubtful if they cover as much as 60 percent of the ground. Around the base of most of the bushes, the soil has accumulated so as to form a slight mound. *Linanthus Parryae* occupies the ground between these mounds, forming a widespread recticulum which is interrupted only by the stream beds or depositions alluded to above. The species ordinarily ranges upward as far as the Juniper belt and terminates at the lower margin of the piedmont where the soil becomes very sandy or alkaline (the approximate limits of its occurrence are shown in figures 1 and 2 by a broken line). As far as can be judged from the nature of the soils and the vegetation which they support, there is nothing to suggest any selection differential in the habitat nor any physical barrier to a free interchange of genes from one end of the area to the other, except for the stream beds. Of such barriers, if any, the arroyo of the Mojave River would appear to be the principal one.

The incidence and amount of rainfall upon the desert varies greatly from year to year. The abundance of *Linanthus Parryae* varies accordingly. In unfavorable years the species may be found only in sparse concentration or abundant only locally; in favorable years it may form an almost continuous sheen of white amongst the bushes where it grows, almost to the exclusion of other annuals. The flowering season of 1941 was exceptional in the amount of rainfall in the area described. As a result, an abundant and practically continuous population of *Linanthus Parryae* was developed from the vicinity of Palmdale to Lucerne Valley (fig. 1, 2). This population was largely, if not wholly, cut off from other similar populations in other parts of the desert by the conformation of the terrain and soils and hence can be treated as a unit.

POLLINATION

The pollination mechanism is as yet unknown but it is presumed from the flower structure that the species is normally insect pollinated. The petals are spread during the day, loosely closed at night. Nevertheless, al-

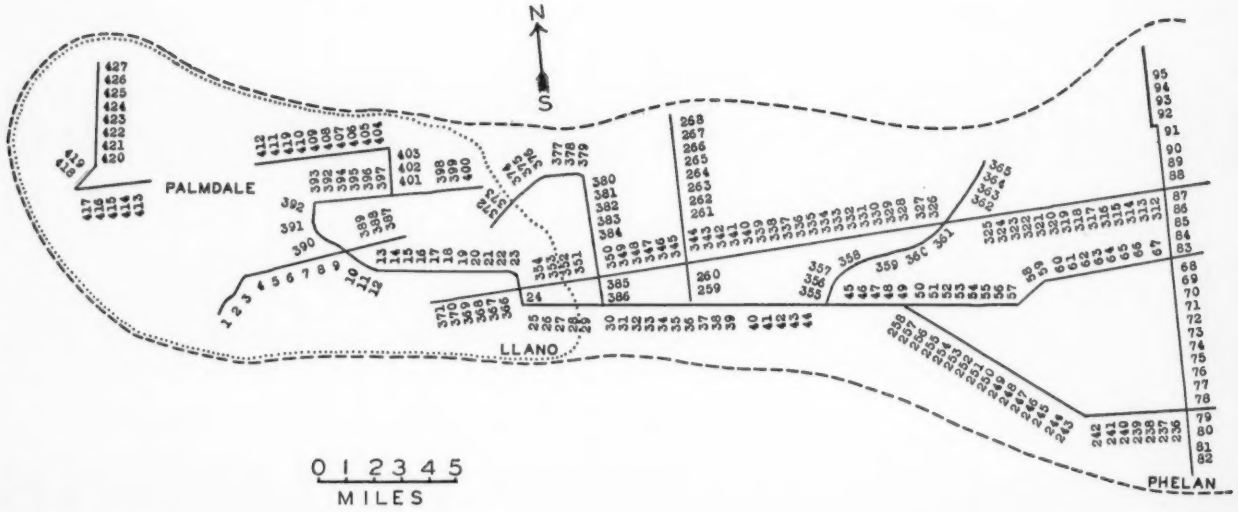

FIGURE 1.—Collecting stations in the western portion of the region in which *Linanthus Parryae* has been examined. Solid lines, the roads traveled; broken line, approximate limit of the occurrence of *Linanthus Parryae;* dotted line, approximate limit of the "variable areas."

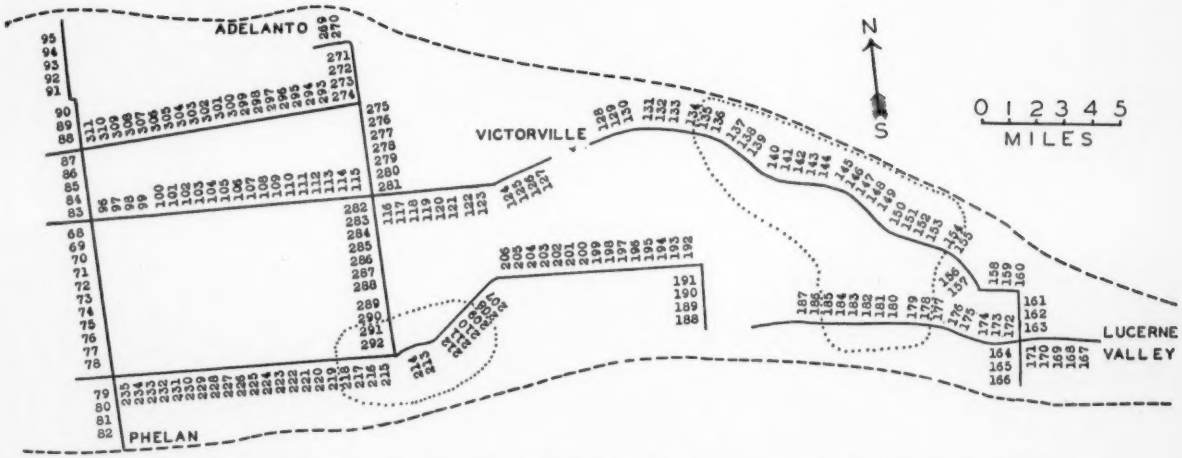

FIGURE 2.—Collecting stations in the eastern portion of the region in which *Linanthus Parryae* has been examined. The significance of the different types of lines as in figure 1.

though thousands of plants in full flower were seen on several successive days, from dawn until sundown, no conspicuous insects were observed upon them. However, in collecting seed at a later date, the following facts were brought to light. The set of seed on a plant varied greatly. Some plants matured no seed whatever, others only occasional capsules, still others most of the capsules. If a capsule matured at all, as a rule most of the potential seeds developed. The capsules which did develop were not necessarily the earliest nor the latest but occurred in any position on the plant. These facts suggest insect pollination and, by inference, cross-pollination. The failure of many capsules to mature suggests the possibility that the insects responsible for pollination were too few in number to cope with the extraordinary abundance of the species in the spring of 1941.

METHOD OF COLLECTING THE DATA

Approximately 200 miles of roadway within the population area were traversed by a party in two automobiles. As can be seen from figures 1 and 2, these roads form a rough grid. A stop was made at each half-mile by the speedometer, and each station, thus determined, was numbered. At each station a transect was run at approximately right angles to the road; within this transect were established four sampling points, two on either side of the road at distances of approximately 20 and 70 paces. Allowing ten paces for the width of the road cut, the four samples were therefore spaced approximately 50 paces from each other (approximately 250 feet apart). The distance between the most remote sampling points at each station was 150 paces or 750 feet.

At each sampling point, a count of white and blue flowered plants was made until a hundred plants were counted within a radius which obviously varied with the density. If the area around the sampling point was entirely white or blue, simple inspection was sufficient to determine this fact. If mixed, a count was made. The total number of sampling stations was 427; the total number of samples 1261; the total number of plants recorded 126,100. The reason why the number of the samples is less than four times that of the stations is that at some of the sampling stations and sampling points no *Linanthus Parryae* was found. The absence of the plants at these points was due either to occasional cultivated areas, as near Little Rock, the stream courses alluded to, or for some reason not discernible.

In the vicinity of the station No. 24 (fig. 1) a transect was run, along which 30 samples of 100 plants each were taken at intervals of five paces (approximately 25 feet). These 3000 plants are not included in the above total of 126,100, but will be referred to later.

At alternate stations from Lucerne Valley westward to Palmdale, de-

terminations of the density were made by counting the numbers of plants enclosed by a rigid wire which had been bent into a frame approximately one square foot. This frame was tossed onto the ground at one of the points of the transect where a sample was taken.

THE DATA

A condensed summary of the data is given in table 1, which is constructed in the following manner. The numbers of the blue flowered plants found in the four samples at each station are indicated consecutively from left to right. Thus, the numbers 39, 58, 57, and 52 at station No. 14 mean that 39 blue flowered plants were found in the sample which was taken 70 paces on the left of the road, 58 in that 20 paces on the left, 57 in that 20 paces on the right, and 52 in that 70 paces on the right of the road. The corresponding numbers of the white flowered plants found, but not cited, were hence, 61, 42, 43, and 48. Zero means that no blue flowered plants were found in a given sample; the sign — means that no plants at all were encountered. At many stations the entire population consisted of white flowered plants. Wherever two or more such stations occur consecutively this fact is indicated in table 1 by the words "all white." This method of presentation has been adopted for saving space, although it conceals the fact that at some of the stations or substations in the "all white" regions the plants were entirely absent.

The thirty samples on the special transect at station No. 24 (see above) are not included in table 1. The numbers of blues in these samples were as follows: 1, 6, 2, 6, 3, 3, 5, 5, 9, 3, 6, 9, 9, 13, 22, 24, 33, 24, 26, 10, 27, 73, 35, 70, 82, 82, 89, 94, 94, 89. This transect, which was made in a singularly homogeneous site, illustrates the nature of the gradient from a "blue" to a "white" area.

The white flowered form was definitely more abundant than the blue flowered form in the region studied. A total of 113,955 whites and 12,145 blues were recorded. However, examination of the data in table 1 in conjunction with the maps in figures 1 and 2 shows that the distribution of the white and of the blue was by no means uniform. The blue flowered plants occurred almost entirely in three disjunct areas, which are indicated by dotted lines in figures 1 and 2. They will be referred to hereafter as the "variable areas." The largest occupies the western extremity of the region around the town of Palmdale and the hamlet of Llano (fig. 1); another lies between Victorville and Lucerne Valley; the third and smallest lies southwest of Victorville (fig. 2). The samples of the variable areas consisted neither entirely nor, with few exceptions, even predominantly of blue flowered plants, and infrequent and often solitary blues occurred outside these areas. However, among the 12,145 blues recorded, only 108 were

TABLE 1

Numbers of blue flowered plants in samples of 100 individuals. The stations at which no plants were found are omitted in this table; large regions in which only white flowered plants were found are marked "all white"; 0—indicates that a given sample consisted of white flowered plants only; the sign — indicates that no plants were found at that point. Further explanation in text.

STATION NUMBER	NUMBERS OF BLUES				STATION NUMBER	NUMBERS OF BLUES				STATION NUMBER	NUMBERS OF BLUES			
1	—	1	0	0	147	4	15	20	25	291	62	47	25	49
3	10	—	1	—	148	—	—	0	0	292	—	—	15	11
4	0	0	—	—	149	3	14	10	10	293–340	all white			
6	—	—	—	100	152	—	—	46	67	341	0	0	5	0
8	55	91	—	—	153	0	0	0	0	342–352	all white			
11	33	60	77	45	154	25	36	—	0	353	0	1	0	0
12	74	8	10	6	156–168	all white				354	19	47	26	15
13	32	—	47	65	170	—	0	0	3	355–366	all white			
14	39	58	57	52	171	0	0	0	0	367	3	0	3	1
19	0	0	0	0	172	—	10	0	0	368	0	0	0	0
20	—	86	72	4	173–174	all white				369	100	99	100	100
21	1	0	0	20	175	0	0	4	2	371	0	—	100	100
22	1	2	0	0	176	—	—	0	6	372	0	1	1	0
23	0	1	4	10	177	0	0	0	0	373	3	1	0	0
24	0	0	—	0	178	—	17	21	33	374–375	all white			
25	100	98	96	100	179	0	0	0	0	376	0	0	2	0
26	60	85	79	85	180	88	92	57	53	377	0	0	0	0
27	0	99	100	—	181	69	100	100	100	378	—	—	0	4
28	100	100	—	—	182	—	—	98	92	379	—	—	0	—
29–35	all white				183	94	100	100	100	380	0	2	0	0
36	0	1	0	0	184	38	—	—	—	383	—	0	0	0
37	0	1	0	0	185	11	15	50	—	384	0	6	0	0
38–40	all white				186–208	all white				385–386	all white			
41	0	0	3	0	209	0	7	0	0	387	54	47	55	79
42–44	all white				210	0	0	9	0	388	43	60	64	30
45	0	0	0	3	211	0	0	0	0	389	65	82	89	57
46	0	0	0	0	212	0	0	0	2	390	44	38	60	67
47	6	0	0	0	213	0	0	5	1	392	—	—	0	0
48–102	all white				214	0	0	0	0	394	—	90	66	98
103	0	5	0	0	215	0	0	50	16	395	54	65	52	59
104–108	all white				216	7	0	5	8	396	37	40	26	50
109	0	1	—	—	217	0	0	0	0	397	55	40	28	20
110–130	all white				218	11	60	62	79	398	36	50	50	62
131	0	0	0	9	219–230	all white				399	22	24	18	13
132–134	all white				231	0	0	1	16	400	26	26	50	—
135	11	15	35	27	232	0	—	0	12	401	50	50	52	72
136	—	0	0	0	233–261	all white				402	42	72	54	50
137	0	0	30	0	262	5	0	0	0	403	14	—	54	56
138–139	all white				263	—	—	0	—	404	69	46	50	63
140	0	1	0	8	264	—	0	1	0	405	35	21	46	59
141	4	0	0	—	266–276	all white				406	47	50	42	79
142	—	17	0	16	277	1	0	0	0	407	40	60	66	64
144	38	—	0	7	278–288	all white				408	—	32	—	—
145	14	4	—	—	289	0	0	1	0	409	—	16	99	100
146	4	4	6	36	290	0	0	0	0	410	28	96	—	—

TABLE 1—*Continued*

STATION NUMBER	NUMBERS OF BLUES	STATION NUMBER	NUMBERS OF BLUES	STATION NUMBER	NUMBERS OF BLUES
411	100 97 — 62	416	— 100 46 48	423	25 50 76 39
412	— — 54 100	418	80 100 98 100	424	12 28 — 9
413	100 100 100 100	419	0 1 88 4	425	2 2 0 0
414	100 100 100 100	421	0 2 6 24	426–427	all white
415	100 100 100 92	422	15 92 62 3		

found outside these areas. Among the above 108, as many as 29 were found at two adjacent stations, Nos. 231 and 232 (fig. 2), perhaps indicating the existence there of a very small fourth variable area. The remaining 79 blue flowered plants were found scattered sporadically in the predominantly white area, mostly as single plants or as groups of fewer than ten plants.

The question may be raised whether or not the delimitation of the variable areas is an arbitrary procedure. To a certain extent it is admittedly arbitrary; indeed, some of the stations within the variable areas yielded only whites, and some blues were found outside these areas. Nevertheless, there is no doubt that the whites and the blues were not distributed at random throughout the entire region studied. Casual inspection of the region en route by automobile was sufficient to suggest this, and anyone who will take the trouble to compare the data in table 1 with the maps in figures 1 and 2 will be convinced not only that the distinction between the variable and the predominantly white areas is a real one, but also that the limits of these areas were approximately as they are indicated on these maps. To illustrate this point, the frequencies of blue and white flowered plants recorded at stations in the eastern part of the Palmdale variable area are shown in sector diagrams in figure 3. There was only a weak tendency for the blues to occur more frequently in the central parts of the variable areas than they did at the margins of these areas. Thus, only whites were recorded at station No. 392 (fig. 3), which is surrounded by stations at which 50 percent or more of the plants were blue. On the other hand, at station No. 28 all the plants were blue, although to the east of it there lies a large area in which only whites were recorded. The boundary of the variable area was drawn between stations 28 and 29, 352 and 353, and 373 and 374. Perhaps one would be equally justified in drawing this boundary west of station No. 372 and between stations 353 and 354 (fig. 3). However, the location of the boundary is fixed within these limits.

FREQUENCY OF SAMPLES WITH DIFFERENT PROPORTIONS OF WHITE AND BLUE FLOWERED PLANTS

Some of the samples in our material contained exclusively white, other samples only blue, and still others were mixtures of white and blue flowered

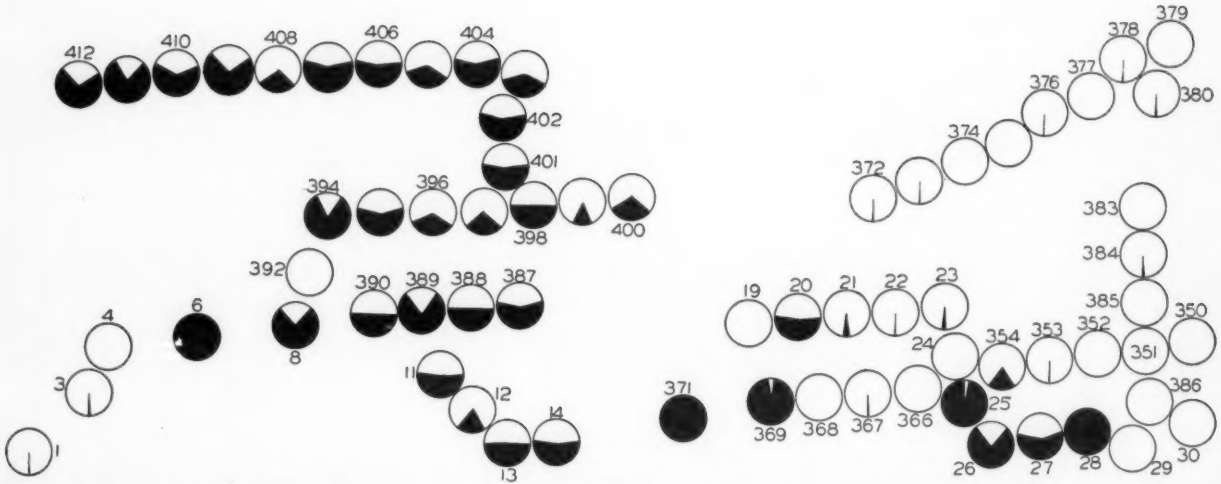

FIGURE 3.—Relative frequencies of the blue and white flowered plants in a part of the region examined (compare fig. 1). Black sectors, blue flowers; white sectors, white flowers. The stations at which no samples were taken are omitted.

plants. In the variable areas outlined above, 349 samples (34,900 individuals, not counting the 3000 individuals recorded on the transect near station No. 24) were taken. Figure 4 shows the frequencies of the samples with different proportions of blue flowered plants. The horizontal axis of the histogram indicates the percentages of the blue in the samples; the lefthand column includes those entirely white and the righthand column those entirely blue. On the vertical axis the percentage frequency of each class of samples is shown. Thus, 98 samples, or 28.1 percent, contain only white; 33 samples, or 9.5 percent, contain one to four percent blue, etc. Disregarding the probably non-significant hump in the middle (lying between 45 and 60 percent), the histogram in figure 4 shows a skewed U-shaped curve. WRIGHT has shown (1931, fig. 7–10; 1937, fig. 1, 4, 7; 1940, fig. 4) that curves of this type characterize the distribution of gene frequencies in populations of small genetically effective size.

The shape of the curve in figure 4 is evidently due to the zero class (all white) and the one hundred class (all blue) being more frequent than the intermediate classes. One would expect such a distribution to arise if the blues were concentrated in one part of the region studied, the whites in another part, and the mixed populations in a transition zone. However, this is not the situation which is found in our material. As stated above, pure white and pure blue populations were encountered in the middle as well as on the margins of the variable areas (cf. fig. 3 and table 1 for illustrations). Examination of correlations (see below) shows that in the variable areas the composition of a population at a given point is independent of that of populations residing 1.5 mile or more away. Under these circumstances, a U-shaped distribution curve probably indicates a restriction of the genetically effective population size in *Linanthus Parryae*.

Among the 912 samples from the predominantly white areas, 887 samples were pure white, 23 samples had from one to ten percent blue, and two samples (stations 231 and 232) 16 and 12 percent blue, respectively. Inclusion of these samples would change the histogram in figure 4 by rendering the curve much more skewed toward the left, without, however, altering the type of the curve. Since four samples were obtained at each station, a histogram was constructed for the average frequencies of blues in populations of whole stations. Such a histogram resembled that in figure 4, but the distribution curve was not clearly U-shaped, owing to the fact that pure blue populations of whole stations were less frequent than in substation samples.

VARIABILITY

Examination of table 1 shows that not only populations of adjacent stations but also samples from the same station may exhibit striking differ-

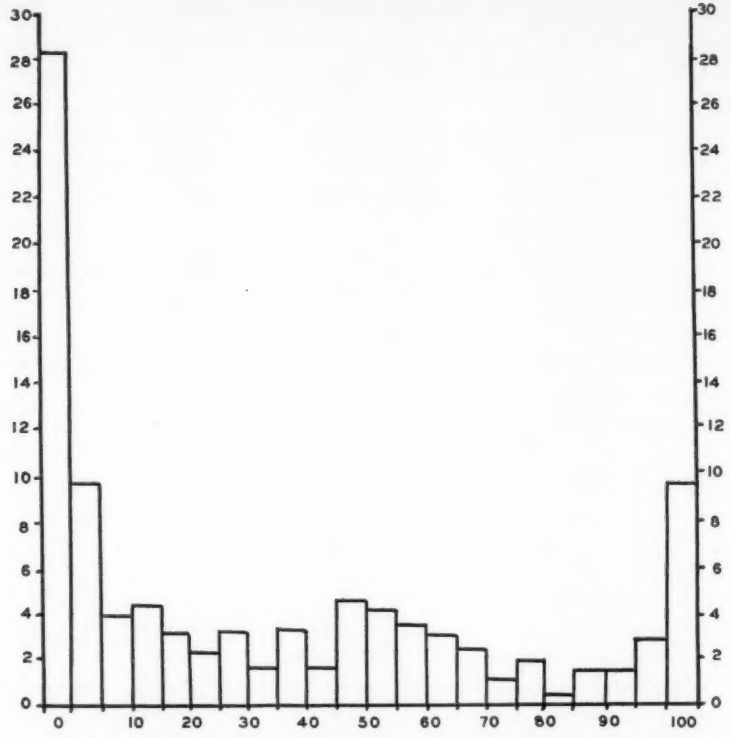

FIGURE 4.—Frequency of samples containing different proportions of blue flowered plants. Horizontal axis, percentage frequency of blues; vertical axis, percentage of samples.

ences in the frequencies of white and blue flowered plants. Thus, the three samples taken at station No. 27 contained 0, 99, and 100 blues, respectively; at station No. 409 samples with 16, 99, and 100 blues were recorded. It is accordingly desirable to know whether or not different samples from the same station resemble each other to a greater extent than do those of different stations. To answer this question, we may compare the intrastation with the interstation mean squares in our material (computed according to SNEDECOR 1934, 1937).

In the western variable area (fig. 1) four samples were obtained at each of 39 stations (table 1). Hence, the material consists of 156 samples or 15,600 plants. The mean squares are as follows:

	Sum of Squares	Degrees of Freedom	Mean Square
Between Stations	162,655.2	38	4280.4
Within Stations	26,083.5	117	222.9

In the two eastern variable areas (fig. 2) four samples are available from each of 25 stations. The mean squares are thus:

	Sum of Squares	Degrees of Freedom	Mean Square
Between Stations	84,959.9	24	3540.0
Within Stations	9,232.5	75	123.1

In all variable areas three samples were taken at each of 18 stations. Computation of mean squares shows:

	Sum of Squares	Degrees of Freedom	Mean Square
Between Stations	50,124.5	17	2948.5
Within Stations	15,714.0	36	436.5

Finally, 15 stations in all varible areas yielded only two samples each. Mean squares in this material are:

	Sum of Squares	Degrees of Freedom	Mean Square
Between Stations	48,172.2	14	3440.9
Within Stations	4,373.0	15	291.5

The F values, that is, the ratios of the larger mean square to the smaller mean square, for the above four fractions of the data are 19.2, 28.8, 6.7, and 11.8, respectively. These values show that the intra-station variances are very significantly smaller than the inter-station variances. It is certain therefore that population samples taken within a station are in general more similar than samples from different stations.

SPATIAL DIFFERENTIATION

The conclusion just stated raises a more general question: to what extent is the similarity (or the difference) between populations of *Linanthus Parryae* a function of the distance between territories inhabited by these populations? To answer this question, a study was made of the correlation between the frequencies of blue flowered plants in populations found at different distances from each other. The following groups of data are useful for this purpose: 30 samples taken at approximately 25 foot intervals in the vicinity of station No. 24 (see p. 323); samples taken at about 250 feet intervals at most of the collecting stations (table 1); and samples taken at different stations, the successive stations being approximately half a mile apart (table 1). Only the data for the variable areas have been included in these correlation tables.

To find how strongly the frequencies of blue flowered plants are correlated in populations found 25 feet apart, pairs of figures which represent the successive samples on the transect near the station No. 24 have been arranged in a correlation table. Similarly, the correlation between populations living half a mile apart has been computed by tabulating pairs of

adjacent samples from successive stations (if the order of the stations be represented as abcde, the pairs ab, bc, cd, de were entered); for distances of one mile, pairs of alternate samples were entered (ac, bd, ce). It is fully realized that the validity of the correlation coefficients in our material may be questioned because the frequency distribution of the different classes of samples shows a striking departure from normality (fig. 3). The justification of using the correlations in this material for our purpose lies in this: that (1) all the correlation coefficients to be compared were derived from distributions which display the same type of deviation from the normal; (2) the correlation coefficients for different distances exhibit a very consistent trend of change (table 2); (3) mere inspection of the data without any computations demonstrates beyond any doubt that populations from

TABLE 2

Correlations between the frequencies of blue flowered plants in populations found at different distances.

APPROXIMATE DISTANCE	$r \pm m_r$	NUMBER OF ENTRIES	BASED ON DATA PRESENTED IN
25 feet	$+0.899 \pm 0.037$	29	page 323
75 feet	$+0.875 \pm 0.065$	27	ditto
250 feet	$+0.817 \pm 0.022$	224	Table 1
750 feet	$+0.723 \pm 0.062$	61	ditto
0.5 mile	$+0.599 \pm 0.066$	95	ditto
1.0 mile	$+0.505 \pm 0.082$	82	ditto
1.5 miles	$+0.115 \pm 0.114$	77	ditto
2.0 miles	$+0.096 \pm 0.109$	84	ditto

closely adjacent localities tend to be more similar than those from more remote localities, thus giving common sense confirmation of the correlation coefficients which actually express a biological regularity present in the data.

Table 2 shows that the populations which inhabited territories 750 feet apart or less tend to have similar frequencies of the blue and the white flowered plants. This conclusion is confirmed by inspection of the data for the 30 samples on the transect at station No. 24. This transect shows a clear gradient in the frequency of blues; at one end of the transect the population is predominantly white, at the other end it is predominantly blue, and a gradual change occurs in the intervening zone. The resemblance of populations found within one-half mile and within one mile of each other is still perceptible. The correlation coefficients for distances of 1.5 and 2 miles are no longer significant.

COLOR VARIATION AND THE ENVIRONMENT

No relation between the composition of populations of *Linanthus Parryae* and the environment in which these populations live is detectable. As pointed out above, the region studied is homogeneous both with respect to physical factors and with respect to plant associations. The boundaries between the variable and the predominantly white areas do not coincide with any perceptible migration barriers. An attempt was made to relate the composition and the density of the populations examined. The outcome of this attempt was negative. The population density in the variable areas fluctuated from less than one to 26 plants per square foot, the average being 9.7 plants; in the predominantly white areas the fluctuation was from less than one to 48, and the average 7.4 per square foot. Correlating the population density and the frequency of blues at different stations within the variable region, a non-significant correlation coefficient ($r = -0.08 \pm 0.16$) was obtained.

DISCUSSION AND SUMMARY

The apparent complexity of the distribution pattern of white and blue flower color in *Linanthus Parryae* can be reduced to a relatively simple scheme. The blue was found principally in three or four "variable areas." Outside these areas the blue was encountered only sporadically, as would be expected if it were introduced there only on rare occasions through mutation or through occasional transport of "blue" pollen or seed. Within the variable areas the white and blue occurred side by side, and the population was differentiated into an extremely fine mosaic of microgeographic races. Pure white and pure blue colonies occurred at distances as small as 500 feet (station No. 27, table 1). Nevertheless, populations found one mile or less apart, resemble each other more than do populations taken at random in the variable areas (table 2).

In the samples from the variable areas (fig. 3) the distribution curve of the frequencies of blue flowered plants resembles WRIGHT's curves for the distribution of gene frequencies in effectively small populations. This resemblance, as well as the extremely fine geographic subdivision of the species in the region studied, furnishes an indication of the agency which is primarily responsible for this mosaic-like subdivision. As shown by WRIGHT (1931 and later work), the variable genes in small populations tend to become stabilized at values of zero and one—that is, tend to be lost or to reach fixation. The distribution curve for such populations consequently assumes a U-like shape which is symmetrical in the absence of mutation, selection, and interchange of migrants among the separate colonies. Introduction of one or more of these factors may cause the U-curve to become

skewed. The curve in figure 4 is definitely skewed toward the left. However, this curve represents phenotype frequencies and not gene frequencies. Since the mode of inheritance of the flower color is as yet unknown, we are unable to compute the gene frequencies in our material. If *Linanthus Parryae* is obligatorily or predominantly crossfertilized, if the difference in color is caused by a single pair of alleles, and if blue is recessive to white, the gene frequency curve would assume a nearly symmetrical U-shape. If blue is dominant to white, the gene frequency curve would be skewed even more toward the left than the phenotype curve shown in figure 4.

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OPPOSITIONAL ALLELES CAUSING CROSS-INCOMPATIBILITY IN TRIFOLIUM REPENS[1]

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IN SEVERAL species in which cross-incompatibility is determined by multiple oppositional alleles, different types of tests have shown that these series of alleles are very extensive. The test yielding the most decisive results and involving the fewest crosses consists of making all possible reciprocal combinations (that is, diallel crosses) between plants which have one *S* factor in common. This procedure was applied first by EAST and YARNELL (1929); they found 15 different alleles among the 32 tested in Nicotiana. A similar test has since been made by WILLIAMS (1937) using red clover, in which 34 out of 40 alleles proved to be different. More recently, WILLIAMS (1941) reported on two other series of red clover plants in which 41 out of 48 and 37 out of 40 alleles, respectively, were different. Likewise, EMERSON (1940) has identified 45 different alleles in *Oenothera organensis*.

A series of oppositional alleles conditioning cross-incompatibility has been found in plants of white clover, irrespective of whether they were self-incompatible, pseudo-self-compatible, or self-compatible (ATWOOD 1940; in press; 1941a). Matings between unrelated plants have very rarely been incompatible, and when selected plants were studied intensively (ATWOOD 1940; in press), seven of the eight alleles that were identified proved to be different. This evidence has suggested that the series of alleles in white clover may be just as extensive as that found in other species.

MATERIALS AND METHODS

Two series of plants were chosen for this study. The first consisted of the ten highest yielding plants of the 64 selections tested in sod (ATWOOD and GARBER, in press). These ten plants arose from seed that had been collected from old, well-established pastures in the following places: Beltsville, Md.; Welcome, Md. (two plants from two different fields, about one-half mile apart); Sparks, Md.; Walpole, N. H.; Ithaca, N. Y.; State College, Pa.; Martinsburg, W. Va.; Blacksburg, Va.; and New Zealand. The second series consisted of 17 plants selected by PROFESSOR H. B. SPRAGUE[2]

[1] Contribution No. 28 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. DEPARTMENT OF AGRICULTURE, in cooperation with the Northeastern States. A demonstration paper of these results was presented at the 1941 Summer Meetings of the Genetics Society of America, Cold Spring Harbor, N. Y.

[2] We are gratefully indebted to PROF. SPRAGUE and his associates for the use of these plants, and to several cooperating agronomists for collecting the seed used in the other series.

from a large nursery (AHLGREN and SPRAGUE 1940) which was examined for potential breeding material. Of these 17 plants, nine were from native seed collected from four pastures in New Jersey. Since several heads had been picked from scattered areas in each pasture, it is probable that each head came from a different plant. The other eight plants in this series came from "commercial" seed supplied by the U. S. DEPARTMENT OF AGRICULTURE. One of these plants was from the "Dixie" blend, three were from Missouri seed, and the other four came from Ladino seed, which had been harvested in Oregon. Seven plants, homozygous, respectively, for seven different *S* factors, were used in this study. They had been derived from four parent plants (ATWOOD 1940; in press) which came respectively from seed or plants collected in Michigan, Pennsylvania, Maryland, and New York. Because of the diverse geographical origins of all plants studied, it is highly improbable that they were closely related.

All of the crosses for this study were made in the greenhouse in the manner previously described (ATWOOD 1941b).

EXPERIMENTAL RESULTS

When each of the 27 selected plants was crossed as a female with a single plant homozygous for one of the previously identified *S* factors(S_1S_1), every mating was found to be compatible, indicating that this particular gene, S_1, was not borne by any of the 27 plants. The resultant seed was used to produce the F_1 generation except in two cases, in which seed from the reciprocal cross was used, since the latter was of somewhat better quality in these cases. Because the same classes were expected in the reciprocal F_1 progenies, use of the reciprocal cross was permissible under the circumstances.

Two intra-sterile, inter-fertile groups were expected in each F_1 progeny. For example: $SxSy \times S_1S_1 \rightarrow SxS_1 + SyS_1$. In order to test for these expected groups, four to eight F_1 plants were intermated within each progeny. Not all possible combinations were made, but enough were completed in every case to be certain of the genotypes. The plants within each progeny were classified into groups before the seed was obtained. Three days after each cross was made, it was noted as being compatible or incompatible on the basis of whether or not the pedicels had reflexed (ATWOOD 1940). In every case, however, this classification by pedicel reflection was confirmed with observations on seed-set. Since the plants continued to flower after the matings within progenies were completed, it was possible to use the same pots for the inter-group crosses and in this way to accomplish as many crosses in one flowering season as might require two seasons using some other species.

The segregations into intra-sterile, inter-fertile groups in the 27 F_1

progenies are shown in table 1. In all but one progeny, two groups were found. It is concluded that 26 of the 27 parents each carried two different alleles—that is, they were heterozygous instead of homozygous for *S* factors. Since only five plants were tested in the progeny yielding only one group, it is highly probable that the parent involved here was also heterozygous. This conclusion appears more certain when one considers the fact that plants homozygous for *S* factors have never been found occurring naturally among the many tested in genetic and breeding studies.

TABLE 1

Segregations into intra-sterile, inter-fertile groups obtained in F_1 populations.

EXPERIMENT NO.	NO. OF F_1 FAMILIES SEGREGATING AS FOLLOWS:						
	4			5		8	Total
	3:1	2:2	5:0	4:1	3:2	5:3	
1st series	2	1	1	2	4		10
2nd series		4		5	7	1	17
Total	2	5	1	7	11	1	27

Since the homozygous parent plant used in the original crosses was of the constitution S_1S_1, and therefore all F_1 plants bore the gene S_1, it was possible by crossing any two groups to test for the identity of the second *S* factor which each of these groups carried. An incompatible mating between any two groups indicated that the alleles being tested were the same, whereas a compatible cross indicated they were different. Similarly, when the homozygous plants were crossed as males with either the parent plants or the two respective F_1 groups, incompatibility indicated the presence of identical genes and compatibility indicated the presence of different genes.

Diallel crosses were made between the F_1 groups within each series, and the homozygous plants were crossed onto the F_1 groups or parents of both series. All possible combinations were completed in each series, and 47 percent of the diallel crosses were tested reciprocally. In general, no difficulty was experienced in distinguishing compatible from incompatible crosses. An average of 0.4 seeds per ten flowers was obtained from the 77 heads, including duplicates, used for incompatible crosses in the first series of plants, whereas the 376 compatible crosses made in this series averaged 36.3 seeds per ten flowers. Likewise, in the second series the average for 176 incompatible crosses was 0.3 seeds per ten flowers, while the average for the 1,023 compatible crosses was 33.1. Whenever an incompatible cross was expected on the basis of the failure of pedicel reflection, an attempt was made to repeat the cross and to make the reciprocal. In every case these

duplicates agreed with the original determination. Similarly, if the first seed-set from a compatible cross was low because of disease, poor pollination, or mechanical injury, the cross was repeated or made reciprocally wherever possible, so that in none of the compatible crosses was the final seed-set so low that classification as compatible or incompatible was uncertain.

All F_1 groups were crossed as females with S_1S_1 to check on possible contaminants. In every case this mating failed, indicating that all F_1 plants bore S_1 and therefore must have resulted from legitimate pollination. With this type of study, it is essential, of course, to know that the groups being crossed do carry one S factor in common.

From the ten parent plants in the first series, 19 F_1 groups were obtained. When diallel crosses were made, only one combination was incompatible. One of the genes from the Charles County, Md., plant proved to be the same as one from the Pennsylvania plant. With this series, all matings with the six homozygous plants were compatible. Altogether, 26 genes were tested and 25 of them (96 percent) proved to be different.

In the second series of plants, six of the diallel matings and one of the matings with the homozygous plants proved to be incompatible. From the 17 parent plants, 34 F_1 groups were obtained, and each of these or their heterozygous parent was crossed with the six homozygous plants. Of the 41 alleles tested in this series, 34 (83 percent) proved to be different. Two genes from seeds collected in the same pasture at Somerville, N. J., were the same, but in every other case the identical genes were found in plants that probably were not closely related. The following list shows the source of the other identical genes: Somerville, N. J.—Washington, N. J.; Somerville, N. J.—Missouri; Somerville, N. J.—Ladino; Missouri—Ladino; Missouri—Ladino; Washington, N. J.—New York.

The last pair on the list were found by testing with a homozygous plant. In no case did more than two genes prove to be the same.

DISCUSSION

The most striking feature of the results reported here is the large number of different allelomorphs found. Except for oppositional factors of the personate type, no other character in any plant or animal is known by the writer in which so high a proportion of the alleles obtained from random sampling has proved to be different. The proportion of different alleles found in these plants of white clover is considerably higher than that found by EAST and YARNELL (1929) in tobacco, but it is about the same as that found by WILLIAMS (1937, 1941) in red clover. EAST and YARNELL concluded that although other S factors may exist, "they are probably few in

number and presumably rare." The higher total number as well as higher proportion of different alleles found in white clover, however, suggest that the total number existing in nature is very much larger than the number already found. There was a somewhat higher percentage of different alleles in the first series of ten plants than in the second series of 17, but this difference is small enough to be of questionable significance. The chance of the parent plants being related was somewhat more remote in the first series than in the second, but one cannot be certain from the present results that this is the only cause of the different percentages.

There seems to be little question that the different factors tested in this material are really multiple alleles. The best evidence is the regular segregation into two groups in each F_1 progeny. EAST and YARNELL (1929) discussed in some detail the nature of these multiple alleles, but they did not know of such large numbers as exist in white clover. The chances for the persistence of local deviations may be especially good in a perennial plant like white clover, and because of its ubiquitous nature, the total number of alleles may have become very large. In no case has any adverse effect been noted on plants bearing a particular allele. Because of this, there may not be any selection against new alleles when they are formed. On the contrary, new alleles would appear to be favored in selection since they would promote cross-fertilization with its resultant hybrid vigor. WRIGHT (1939) has given methods for estimating the number of alleles in a population. From this it can be inferred that the total number in white clover is large.

With these plants of white clover, the difference between compatible and incompatible crosses was distinct in every case, so that little difficulty was experienced either in separating the two groups within each F_1 progeny or in deciding on the total number of different genes. Difficulties were encountered by EAST and YARNELL (1929) in classifying within families, but their families were much larger than those used in white clover. Of major assistance in all this work were the facts that the degree of pedicel reflection was an accurate measure of how well seed was setting and that the same plant could be used for several matings. If there was any question about compatibility, the cross could be repeated immediately to check on the cause of the questionable results.

The most important application of these results appears to be that cross-incompatibility between unrelated plants of white clover should be expected very rarely. In any breeding program, it is important, of course, to know the degree of free intercrossing which is to be expected. Crossing within families of white clover will necessarily result in some incompatibility, but the more remote the relation, the less often will incompatibilities be found.

SUMMARY

Two series of *Trifolium repens* plants (ten and 17 individuals, respectively) were compatible as females with a plant homozygous for oppositional alleles (S_1S_1). In 26 of the 27 resultant F_1 progenies of four to eight plants each, two intra-sterile, inter-fertile groups were found, while in the other progeny only one group was found.

Within each series, diallel crosses were made for all F_1 groups, and each F_1 group or its heterozygous parent was crossed as a female with six plants, homozygous respectively for six other alleles. Incompatible matings indicated that the two alleles being tested were identical. Of the 26 alleles tested in the first series, 25 (96 percent) proved to be different, and in the second series 34 (83 percent) of the 41 were different.

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STUDIES ON AN ANOPHTHALMIC STRAIN OF MICE.

III. RESULTS OF CROSSES WITH OTHER STRAINS

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INTRODUCTION

EYELESSNESS has been found in individuals throughout the vertebrates, and blind species are known in almost all classes. This strain of eyeless mice is more extreme in eye reduction than any blind species. It provides an opportunity for the study of factors affecting the expression of this character which in turn may help in understanding the phylogeny of blind species.

With selection and inbreeding, the hereditary minor factors (modifiers) have become practically homozygous, thus making possible an analysis of the heredity of eyelessness without the confusion of unpredicted modifiers. Two methods of analysis of the mode of inheritance of this eyelessness have been undertaken. The present paper deals with the inheritance when the modifiers are not the same—that is, crosses with different strains of mice. Another method involves a control of the modifiers, made possible by a mutation toward normal eyes in the inbred eyeless strain. This study is in progress at the present time.

The embryology of this anophthalmia has been shown to be fundamentally an inhibition of the growth of the optic vesicle at ten days or earlier, just after the normal formation of the vesicle (CHASE and CHASE 1941). This inhibition may prevent the vesicle from forming a cup; or, more often, a small, irregular cup forms, usually too far from the surface to induce a lens. Whatever is present by 13 days usually grows at a normal rate with the remainder of the embryo but is too late to organize into a normal functional eye. The variations result in the adult in (1) complete anophthalmia with no eye remnants but with normal lids, orbits, and conjunctivas and large lacrimal glands (actually Harderian glands) filling the normal eye sockets; (2) a "very small" eye of only a pigment mass or with a little abnormal retinal tissue in addition; (3) a "small" eye with lens and abnormal retina; or (4) a "medium" eye with normal proportions. The medium eye can be seen between the lids; small and very small eyes cannot.

Reproduction in this strain is not affected (E. B. CHASE 1941). Lack of eyes and optic nerves are apparently no detriment to normal reproductive behavior in these animals.

DESCRIPTIONS OF STRAINS USED

Birth records of the eye condition were taken because variations below "medium" in size are not seen externally in the adult. Postmortem examination confirmed the birth records. The grades of eyes used for all mice in the colony are (1) eyeless, (2) "very small," a mere speck of pigment, (3) "small," (4) "medium," (5) normal. In all the strains of mice used, asymmetry is very common, with a high degree of dissimilarity between the left and right eyes. Table 1 presents for the strains and some crosses these asymmetries, the effect of sex, the correlations between left and right eyes, littermates, sibs, parents and offspring, and age of parent.

Strain B. *CC AA bb dd SS*. This is the anophthalmic strain. The original animals (from strain R) were obtained in 1938 from DR. C. C. LITTLE of the ROSCOE B. JACKSON MEMORIAL LABORATORY at Bar Harbor, Maine. Reduced eyes were noted in offspring from a wide cross, and there was subsequent selection by LITTLE for this character. Seventeen successive generations (12 at Illinois) of brother-sister matings have now been made. This strain consists of 0.0 percent normal on both sides, 9.0 percent intermediate (for instance small left and eyeless right, or small on both sides, etc.), 2.7 percent very small (for both eyes or very small for one and eyeless for the other), and 88.3 percent completely eyeless.

In strain B the left eye tends toward normality more than does the right, the male tends in this direction slightly less than does the female. A correlation of +.38 exists between the left and right eyes, indicating that although there is much asymmetry the eyes do not vary completely independently. Littermates with a correlation of +.18 and sibs with a correlation of +.09 suggest that much of the variation is due to non-hereditary "modifiers" common to littermates (as intra-uterine environment at the particular time). Some of this is apparently an effect of the age of the mother ($r = -.06$), since with increasing age of mother there is less tendency to deviate from complete eyelessness. The age of the father is also a possible factor, however, because in inbreeding the strain, littermates have usually been mated, and there are too few cases of sibs of different ages being mated to give a significant correlation to distinguish between effect of mother and effect of father. The +.09 parent-offspring correlation and +.09 sib correlation indicate that little variation is due to hereditary modifiers.

No significant difference with respect to the frequency of anophthalmia (about 90 percent) and microphthalmia (about 10 percent) exists between the present mice, now in the 12th generation of successive brother-sister matings at Illinois, and those of the first five generations which were used in the original crosses with other strains. Only pure anophthalmics are

TABLE 1

Asymmetries and correlations in strains and some crosses.

STRAIN	ASYMMETRIES: PERCENTAGE EYES NORMAL (OR MERELY NOT EYELESS FOR STRAIN B)				ASYMMETRIES: PERCENTAGE OF INTERMEDIATE ANIMALS WITH LEFT EYE GREATER THAN, EQUAL TO, OR LESS THAN RIGHT			CORRELATIONS				
	♂ LEFT	♂ RIGHT	♀ LEFT	♀ RIGHT	L>R	L=R	L<R	LEFT AND RIGHT EYES	LITTERMATES*	SIBS*	PARENTS AND OFFSPRING*	AGE OF MOTHER (AND FATHER)
B	6.2	4.0	7.6	5.2	55.7	14.8	29.5	+.38	+.18	+.09	+.09	−.06
C_{57} Black	100.0	98.8	96.8	93.2	76.7	6.7	16.7	+.45	+.08	−.01	−.02	−.00
K	100.0	100.0	100:0	100.0								
L	100.0	100.0	100.0	100.0								
H	100.0	100.0	100.0	100.0								
F_2, $(B \times C_{57}Blk)^2$	80.2	76.6	78.0	67.8	64.4	27.4	8.2	+.80				
BX, $((B \times C_{57}Blk) \times B)$	37.8	35.0	28.6	24.7								
BX, $((B \times K) \times B)$	33.9	30.6	28.6	26.2								
F_2, $(B \times L)^2$	46.5	45.5	43.0	43.3								
BX, $((B \times L) \times B)$	38.6	40.9	30.6	33.3								
F_2, $(B \times H)^2$	42.2	42.2	46.1	46.1								

* Only females for C_{57} Black.

used in the brother-sister matings in continuing the strain, and yet frequencies remain the same, indicating practically complete homozygosity of modifiers.

Strain C57 Black. *CC aa BB DD SS*. This strain was obtained in 1938 from DR. W. L. RUSSELL of the ROSCOE B. JACKSON MEMORIAL LABORATORY, Bar Harbor, Maine. It consisted of animals from his sublines 6 and 10, and these sublines have been continued separately by successive brother-sister matings for 12 generations here at Illinois and were inbred for at least 20 generations previously. The small differences in the sublines noted first by RUSSELL (personal communication) made an insignificant difference in the results of the crosses with strain B, and so the sublines have been grouped under the general strain heading of C57 Black.

Ninety-five and seven tenths (95.7) percent of the animals have normal eyes on both sides, 4.3 percent have intermediate eyes, and 0.1 percent have no eyes or eye remnants (birth records). In the intermediates there is a strong tendency for the left eye to be normal or more nearly so than the right—the same tendency, but apparently stronger, that was seen for strain B. Sex is a much more important differentiating factor in this strain than in B, since only 0.5 percent of the males are without two normal eyes in contrast to 8.3 percent of the females (table 1 and table 2). The correlation of left and right eyes is +.45, which is rather similar to +.38 in strain B. There is some littermate correlation (+.08) but no correlation between sibs (−.01) or between parents and offspring (−.02). These latter correlations were done only for the females in the strain, since intermediate males are too rare. There is no correlation apparently with age of parents, unlike strain B. The parent-offspring and sib correlations indicate that the strain is highly homozygous with no variations due to hereditary differences in the offspring.

Strain K. *cc aa BB DD SS*. This strain is from the Aka stock. Of more than 500 individuals that have been observed carefully at birth, all have had normal eyes on both sides. The strain is considered highly inbred, but rigorous adherence to brother-sister matings was started only at the time the original crosses were made.

Strain L. *cc AA bb DD SS*. This strain is derived from the Swiss stock. Of more than 900 animals examined carefully at birth, all have had two normal eyes. At the time of the original crosses with the anophthalmic strain B, this strain L had not been subjected to any planned inbreeding.

Strain H. *CC aa bb DD ss*. In more than 1000 individuals examined in this spotted brown strain all eyes have been normal. Crosses with B were made in the early generations of inbreeding.

Therefore, at the time original crosses were made, homozygosity for factors affecting eye development was complete for C57 Black sublines,

practically complete for strain B, and probably only partially complete for K, L, and H.

RESULTS OF CROSSES

An important point is that the F_1 is 100 percent normal with respect to eyes in all the crosses with the anophthalmic strain. This indicates that many of the modifiers are different. This is especially interesting for B×C57 Black, since both strains produce intermediates.

TABLE 2

Percentage of animals with normal eyes, intermediate eyes, and no eyes.

		PERCENTAGE			TOTAL NUMBER
		NORMAL	INTER-MEDIATE	EYELESS	
Strain B	total		9.0	91.0	1054
Strain C57 Blk	total	95.7	4.3	0.1	2200
	♂	99.5	0.5		1060
	♀	91.7	8.0	0.3	1140
F_1, (B×C57Blk)		100.0			219
F_2, (B×C57Blk)^2		70.4	19.6	10.0	608
BX, ((B×C57Blk)×B)		59.5	24.7	15.8	158
2BX eyl*, (((B×C57Blk)×B) eyl×B)				100.0	33
2BX, (((B×C57Blk)×B)×B)		29.7	36.0	34.2	150
3BX, ((((B×C57Blk)×B)×B)×B)		29.0	36.7	34.2	38
Strain K	total	100.0			>500
F_1, (B×K)		100.0			54
BX, ((B×K)×B)		57.7	23.1	19.2	52
2BX, (((B×K)×B)×B)		46.7	23.3	30.0	30
Strain L	total	100.0			>900
F_1, (B×L)		100.0			27
F_2, (B×L)^2		88.7	5.9	5.4	407
BX, ((B×L)×B)		70.0	15.0	15.0	40
Strain H	total	100.0			>1000
F_1, (B×H)		100.0			8
F_2, (B×H)^2		88.7	6.1	5.2	115

* This backcross is eyeless×eyeless instead of the usual normal×eyeless.

In the F_2, F_3, and backcross generations there are all possible variations and asymmetries. Table 1 presents some data on these asymmetries. Males tend to have a higher percentage of normal eyes and a higher grade of intermediate eyes than females except in the cross with H. In B×C57 Black where the latter strain had this sex difference strongly pronounced, the effect is diminished, as might be predicted. In both sexes the left eye tends to be more normal than the right eye in the crosses except with L and with H. In B×C57 Black, where this left and right difference is observed in the two parent strains, the effect is also pronounced. In the F_2 (B×C57

Black) intermediates (see table 1) there is a very strong tendency for the left eye to be equal to or greater than the right, and this tendency is stronger than would be predicted from the variations in the two parent strains. The correlation of +.80 between left and right eyes in this F_2 is high, as might be expected since hereditary as well as non-hereditary modifiers are here involved.

Table 2 is a condensed table of the results of the crosses between the anophthalmic strain B and the four other strains. Most of the crosses have the male as the anophthalmic parent. Some reciprocal crosses were made but have shown no significant difference in the eye variations and have therefore been combined in this table with the others. Animals which have eyes of the very small category on both sides or very small on one side and eyeless on the other are here combined with the completely eyeless animals. Justification for this procedure is obtained from the embryological observation that very small and eyeless are rather similar with no lens, whereas small, medium, and normal always have a lens. Further justification is found in the fact that in the F_3 and F_4 generations very small and eyeless give similar breeding results. These results are unlike those obtained from small or medium.

B×C57 Black. Generations beyond F_2 (F_3 through F_9) have been observed for the B×C57 Black cross and indicate great differences in the array of hereditary modifiers. Selection toward normal eyes or toward eyelessness is quickly effective. These generations show what has already been shown by the F_1 generation—namely, that the strains differ considerably in the modifiers possessed. Repeated backcross generations, however, are of much more value in the study of the inheritance of eyelessness because the modifiers are much more controlled. In the second backcross generation, for instance, we can say that approximately 7/8 of the modifiers are of the type present in strain B, the anophthalmic strain.

The data in table 2 indicate a difference of one major factor with dominance. Modifiers lower the expected percentage of normals and raise the expected percentage of completely eyeless. The second backcross generation from an eyeless segregant of the first backcross generation mated back to the original eyeless parent gives 100 percent eyeless or very small. Contrast this with a littermate having normal eyes mated back to the same original parent and producing only 34.2 percent eyeless or very small. A major factor difference is clearly involved. The second and third backcrosses are identical, showing again that the differences in the individuals are not solely differences in the arrays of modifiers, since one is 7/8 of strain B and the other 15/16. The first backcross is somewhat puzzling, since it deviates from the theoretical in the opposite direction from the F_2 and the second and third backcrosses. There is an excess of normal-eyed

individuals. The probability of such a deviation from the theoretical 50:50 is .01, which might be considered as probably significant. However, the deviation from the other generations (2BX, 3BX, F_2) has a probability of <.00001, which is extremely significant. The first backcross definitely does not conform but still does not invalidate the remainder of the evidence, which indicates one major factor difference plus modifiers.

B×K. As in the cross with C57 Black, one major factor showing dominance of normal eyes is indicated.

B×L. Although strains K and L both have normal eyes in all individuals, there is a striking difference displayed in the results of crosses with strain B. The actual F_2 percentages for 407 individuals are 88.7 percent normals, 5.9 percent intermediates, 5.4 percent eyeless. These results might be explained as due to one major factor difference showing dominance for normal eyes. The divergence from the expected F_2 75:25 and BX 50:50 would be due more to strong modifiers toward normality from strain L than counter-balancing modifiers toward eyelessness from strain B. On the other hand the F_2 data approach a theoretical 93.75:6.25, which is expected if two major factors are involved and both dominants are necessary for normal eyes. The backcross of 70 percent, 15 percent, and 15 percent would fit into this two factor scheme equally well (theoretical 75:25).

B×H. The F_2 generation is almost exactly like that of B×L, and again there are the two hypotheses suggested: two major factors with both dominants necessary for normal eyes and strong minus modifiers from B; or one major factor with dominance of normal eyes and strong plus modifiers from H.

DISCUSSION

The anophthalmic strain of mice differs from the other strains with which it was crossed by one major factor (possibly two for some strains) and many minor factors. The symbol *ey* has been suggested (CHASE 1941) for the recessive major gene responsible for eyelessness in this anophthalmic stock. The committee on mouse genetics nomenclature (DUNN, GRÜNEBERG, and SNELL 1940) have suggested that a gene symbol be the initial letter of the character which differs from wild type, providing such a letter is not already used as a symbol. The present writer, however, suggests that *ey* be the symbol instead of *e*, since (1) *e* has long been used with some other rodents, as the guinea pig, for the yellow coat color in the extension series and since (2) *ey* is the symbol employed for the eyeless character in Drosophila. Gene *ey* has shown no linkage with *c*, *a*, *b*, *d*, or *s*. Evidence of an additional major factor in some crosses is not yet sufficiently conclusive to warrant a symbol of *ey-2*.

There is a considerable amount of non-hereditary variation, some of a nature common to littermates but not to sibs, and some of a nature com-

pletely individual. Non-hereditary variations are frequently observed in genetic studies and can be classified as due to environmental (external and internal) factors; but the exact nature of the cause of these variations is usually unexplained. Two animals of the inbred line, for instance, will have identical hereditary constitutions,—that is, transmit alike,—but one may be completely eyeless and the other have a small left and a very small right eye. Part of such variations may be found to be common to littermates, and part of this in turn may be attributed to age of parents. In this anophthalmic strain, for instance, the older the mother (or father) the greater is the frequency of completely eyeless offspring. A much larger proportion of non-hereditary variation is individual, not even common to littermates. The remainder of the variation is even more localized, producing in this case the dissimilarities between the left and right eyes of an individual. That there is a difference in the inhibition of eye vesicles on the two sides can be seen from the study of the embryology of this region (CHASE and CHASE 1941), but it is not known what conditions cause the inhibition and the variations of it. The major hereditary factor, the minor hereditary factors, and all the non-hereditary factors produce in some manner the different degrees of inhibition of eye vesicles which result in the eye variations in the adult.

In the cases of microphthalmia and its extreme expression, anophthalmia, which occur sporadically in many colonies of laboratory rodents, the inheritance has been difficult to determine because of the extreme variability of expression. In a study on anomalies in Wistar rats (KING 1931), anophthalmia was considered to be a trait not inherited, and microphthalmia was considered to be inherited probably as a recessive but with complications and more than one factor involved. Hereditary microphthalmia was reported as occurring to some extent in one line of a stock of Wistar albino rats (HAIN 1933) and as probably recessive. Anophthalmia or microphthalmia showed irregular inheritance in a colony of rats (HOFMANN 1912) in which usually only anomalous females produced any anomalous offspring. In the guinea pig an eye defect varying from drying of the cornea to microphthalmia was found to be due probably to one major factor incompletely recessive to normal, with numerous plus and minus modifiers (LAMBERT and SHRIGLEY 1933).

The results of the crosses of the anophthalmic strain B and the other strains recorded in this paper may help to clarify some of the results with these other colonies. The trait may be due entirely to non-hereditary variations as in approximately five percent of the highly inbred C57 Black sublines of mice, or it may be due to a factor such as *ey* homozygous, with various arrays of hereditary minor factors and the ever-present non-hereditary variations. Some stocks, similar to K, L, and H in mice, have

hereditary *Ey* and minor factors sufficiently above the threshold for normal eyes that the range of non-hereditary variations never results in an animal with abnormal eyes. Strain K is definitely nearer the threshold than L or H as evidenced by the results of the crosses reported in this paper, yet K (100 percent normal) would be considered more like L and H than like C57 Black. A definite selected and homozygous strain like the anophthalmic B is first necessary for accuracy in determining the mode of inheritance of a character so variable in expression.

A point of evolutionary significance is that selection of one or two major factors and some modifiers has produced in only a few generations a stock of mice more extreme in their average lack of eyes than any of the blind species of vertebrates. In fact only one major mutation made homozygous is sufficient apparently in some individuals from B×C57 Black, to produce 100 percent microphthalmics and anophthalmics. These have more extreme reduction of eyes on the average and no greater variation than individuals among the marsupial moles, *Notoryctes typhlops* (SWEET 1906), the most extreme microphthalmia found as a blind species in nature. In view of the ease with which extreme eyelessness can be derived in a few generations of mice, the common assumption that many millions of years must have been required to bring about a change as drastic as a blind species seems unnecessary. Just as albinism is the result of a simple failure to form pigment, microphthalmia is the failure of the eye vesicle to grow sufficiently at a particular stage of embryology. Yet in evolutionary and taxonomic terms albinism is considered as only a simple character, whereas the reduction of eyes is considered as a complicated character and alone sufficient, for instance, to justify the designation of a separate species for a group having this trait. *Anoptichthys jordani*, the Mexican blind cave fish of family Characidae (HUBBS and INNES 1936), is placed in a separate genus solely because of a moderate reduction in the eyes. Millions of years may well have been involved in the histories of many blind species since the time of the last common ancestor with the present similar species with normal eyes. But eye reduction *per se* need not denote antiquity.

SUMMARY

Inheritance of eyelessness in a strain of mice is studied as it is evidenced from crosses with other strains, involving F_1, F_2, F_3, and successive backcross generations. The five strains used are described as to source, eye characteristics, and homogeneity.

Asymmetry of eye is found and considered in the anophthalmic strain among the ten percent not completely eyeless (determined at birth and by post-mortem examination) and in the offspring of the various crosses made.

Crosses gave in all cases 100 percent normal F_1's indicating that, even with C57 Black which slightly overlaps the range of the anophthalmic strain B, at least some of the hereditary minor factors are different in the two strains. Two strains, C57 Black (95.7 percent normal) and K (100 percent normal), showed a difference of one major factor from anophthalmic B; two other strains, L and H (both 100 percent normal) showed a difference of one major factor with very strong modifiers not found in C57 Black and K, or possibly two major factors, the double recessive being necessary for eyelessness.

The gene symbol *ey* is suggested for the one certain major factor, homozygous in the anophthalmic strain.

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FREQUENCY OF WING-BEAT AS A CHARACTER FOR SEPARATING SPECIES RACES AND GEOGRAPHIC VARIETIES OF DROSOPHILA

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INTRODUCTION

THE work of DOBZHANSKY on the variations in natural populations of *Drosophila pseudoobscura* has demonstrated an extensive array of physiological differences between Races A and B of this species and between different geographical samples within each race. It has recently become possible to inquire more intimately into these physiological differences by means of the technique for measuring the frequency of wing-beat during insect flight, as worked out by CHADWICK (1939a) and WILLIAMS (1941).

Wing-beat frequency expresses the intensity of the flight activity. Since flight is dependent on the interaction of most of the physiological systems of the animal, we would expect changes in these systems to be reflected in the wing-beat frequency. Under standard experimental conditions it has been possible therefore to compare in terms of this physiological variant many taxonomic categories within the genus Drosophila.

A major objective was the study of the genetic mechanism controlling wing-beat frequency. This was accomplished by making the proper crosses between species or strains having different wing-beat frequencies. Finally, an attempt was made to ascertain how the genetic mechanism achieves this control.

The authors wish to acknowledge the kindness of PROFESSOR DOBZHANSKY in furnishing most of the stocks and many valuable suggestions.

MATERIALS AND METHODS

A description of the stroboscopic technique for measuring wing-beat frequency has been given by CHADWICK (1939b) and need be only briefly described. The stroboscope consists of a neon-filled tube arranged in a tuned circuit so that the flash-frequency is instantly adjustable over a range of 600 to 14,000 flashes of light per minute. Since there is no afterglow, any cyclical movement, such as that of the insect wing, can be brought to an apparent standstill when the flash-frequency of the instrument is tuned to equal exactly the frequency of the movement. The calibrated scale then gives the frequency directly.

The animals were fastened to a paper strip and wing-beat frequency determined in moist air during short flights stimulated by removal of a platform from under their feet. In every case, the measured wing-beat frequency

was the maximum frequency occurring during the first few seconds of flight. Readings were taken at 20 second intervals, and 20 measurements were made on each individual.

All experiments were carried out at 20.0±0.1°C on virgin female animals 72±2 hours old. These animals were the offspring of single pair matings and were raised under uniform food conditions in a room having a constant temperature of 20.0±0.5°C. About 25 animals of each geographic variety or

TABLE 1

Frequency of double wing-beats (in thousands per minute) of the two races of D. pseudoobscura and of the closely related species D. miranda.

SPECIES	RACE	NO. OF ANIMALS FLOWN	FREQUENCY OF WING-BEATS (THOUSANDS PER MINUTE)	LOCALITY WHERE STRAIN ORIGINATED	APPROXIMATE ALTITUDE OF LOCALITY
D. pseudo-obscura	A	32	11.47±0.04	Willapa Bay, Wash.	sea level
	A	26	11.45±0.05	Perpetua, Ore.	sea level
	A	29	10.48±0.10	Kaibab, Ariz.	10,000 ft.
	A	29	10.81±0.04	Prescott, Ariz.	5,400 ft.
	A	27	10.42±0.06	Quezaltenango, Guatemala	5,000 ft.
	A	Average	10.93±0.05		
	B	37	10.14±0.07	Willapa Bay, Wash.	sea level
	B	25	10.54±0.07	Perpetua, Ore.	sea level
	B	37	10.43±0.06	Yolla Bolly, Calif.	7–9,000 ft.
	B	25	10.72±0.05	Stony Creek, Calif.	6–7,000 ft.
	B	Average	10.46±0.04		
D. miranda		18	9.27±0.08	Cowichan Lake, B.C.	500 ft.
		21	9.56±0.06	Orick, Calif.	sea level
		26	9.95±0.07	Mt. Whitney, Calif.	7,000 ft.
		Average	9.59±0.06		

species were flown. Since coefficients of variability for all the types studied were within 1.8–4.0 percent, it is clear that samples of this size were quite sufficient for rather rigorous analysis.

The stocks of *D. pseudoobscura* were each descendants of a single fertilized female captured at the stated locality. Most of the stocks had less than a year of laboratory existence since their collection by PROFESSOR DOBZHANSKY.

WING-BEAT FREQUENCY IN D. PSEUDOOBSCURA AND D. MIRANDA

In DOBZHANSKY'S (1941) book extensive evidence is presented in regard to the genetic and physiological differentiation of geographic strains of *D. pseudoobscura* and its relative *D. miranda*. One would expect from this

evidence that differentiation of the various populations would have occurred also in regard to wing-beat frequency. This expectation is confirmed and demonstrated in table 1. The three geographic strains of *D. miranda* show highly significant differences. In Races A and B of *D. pseudoobscura* all geographic strains differ significantly from some others but not from all others. In terms of wing-beat frequency the Race A strain from Willapa Bay, Washington, seems to be identical with the Race A population some 200 miles farther down the coast at Perpetua, Oregon. However, the Race B populations from these two localities, as represented by our stocks, are significantly different. The average difference between all the tested strains of Race A and Race B is only 470 double wing-beats per minute, but this difference is 7.34 times its standard error and is therefore highly significant.

Correlation with the number of teeth in the sex combs

The picture of the differences in wing-beat between Races A and B and their geographic variants is strikingly similar to the situation in regard to the number of teeth in the sex combs of the males demonstrated by MATHER and DOBZHANSKY (1939). They showed the number of teeth to be higher in Race A than Race B, though an occasional geographic strain of Race B would have a higher number of teeth than one or more of the geographic strains of Race A. Yet there is no question that on the average Race A has a significantly higher number than Race B. So it is with wing-beat frequency.

Correlation of wing-beat frequency and habitat

In table 1 the approximate altitudes of the various localities from which the animals were collected are given. No correlation between wing-beat frequency and altitude is immediately apparent.

The geographic localities in table 1 are arranged in order, starting in the north and progressing southward in each of the three groupings. The more southern latitude may not be warmer, necessarily, because of differences in altitude. Race A wing-beat frequency seems to decrease southward while Race B seems to increase southward. In *D. miranda* there is a clear indication of increased wing-beat in the southern populations. Thus Race B and *D. miranda* are in conformity, while Race A disagrees with them. Considering only the seeming trends *within* the three groups there seems to be a lack of correlation between latitude and wing-beat frequency. But, taking the averages for the three groups, we get a strong relationship between habitat and wing-beat frequency. It is known from early work of DOBZHANSKY (1937) that *D. miranda* prefers a colder climate than Race B and that Race B does best at a lower temperature than the optimum for Race A. This ecological relationship is in perfect agreement with the wing-beat frequency, which increases markedly with the optimum temperature

progression from *D. miranda* through Race B through Race A. We have once again a picture similar to that of the teeth in the sex combs in which individual populations from particular localities may fluctuate considerably and give false conceptions of relationships which become clear when the grand averages for the racial or species groups are examined.

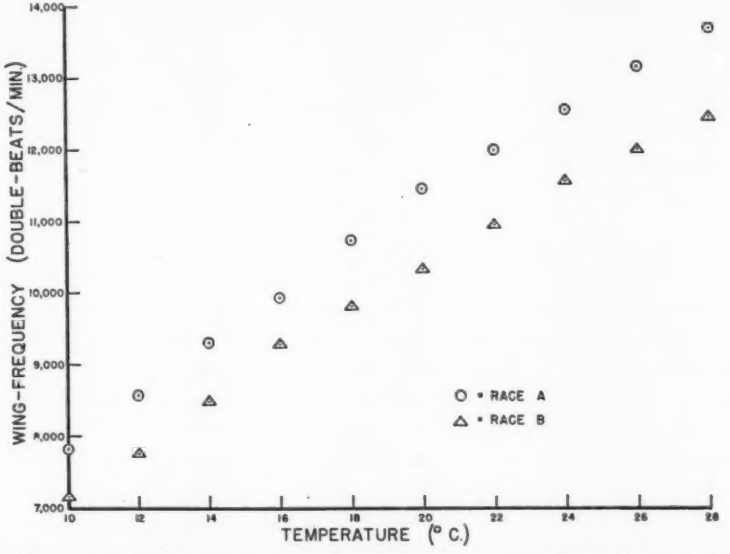

FIGURE 1.—Effect of environmental temperature on the wing-beat frequencies of Races A and B of *Drosophila pseudoobscura* (Willapa Bay). Each point for Race A is the average of eight individuals and for Race B of seven individuals.

The correlation between faster wing rate and a warmer habitat was tested by observations on a second species complex. *D. athabasca* is an Alaskan and northern species rather closely related to, and produces hybrids with, *D. azteca*, which ranges south to Guatemala. Clearly, *D. athabasca* lives in a colder habitat and, if in agreement with the situation in the *D. pseudoobscura-D. miranda* group, should have a slower wing-beat frequency than *D. azteca*. The confirmation of the correlation was remarkable. *D. athabasca* (locality, Gravina, Alaska) flew at 9.25 ± 0.09 thousand double beats per minute while *D. azteca* (locality, Deer Creek, California) was very fast with 11.26 ± 0.03 thousand double beats per minute. The fly with the warmer habitat had exactly 2,000 more beats per minute than the one from the colder region. *D. affinis* occupies an intermediate geographic range between *D. athabasca* and *D. azteca*. The average wing-beat frequency for two *affinis* strains was 10.42, which is likewise intermediate.

DR. WILSON STONE has pointed out a comparable case in the *D. virilis* group. *D. montana*, which lives at high altitudes and in a cold habitat, has extraordinarily large wings when compared with *D. texana*, a warm habitat relative. The generalization is true that, of these groups at least, the race or species living in the warmer habitat has the higher average wing-beat frequency.

Temperature relationship

Since wing-beat frequency of the individual fly varies markedly with changes in environmental temperature, it appeared possible that the wing-beat differences observed between Races A and B at 20.0°C might be due to differences in their responses to temperature. To test this possibility, the effects of varying temperature on the wing-beat frequency of Races A and B were studied. It was found (fig. 1) that the shapes and slopes of the two curves were highly similar, thus showing that the observed difference in wing-beat frequency at 20.0°C was not due to physiological differences between the two races in their responses to temperature.

THE GENETICS OF WING-BEAT FREQUENCY

The large difference in wing-beat frequency between Willapa Bay B and Perpetua A of 1,300 beats per minute provides an opportunity to study the inheritance of a physiological character which can be measured precisely. It should be realized, however, that the conception of the genetics of a physiological character is usually an abstraction. A "physiological character" rests ultimately upon the total effect of various morphological, chemical, and other factors. If the wing-beat frequency depends upon such a composite of groups of factors, then the inheritance should be of the multiple factor type. Such characters usually lack dominance and give a typical picture of intermediacy of the F_1 and backcross generations.

Table 2 shows this to be true of the inheritance of wing-beat frequency. The F_1 and backcross generations had wing-beat frequencies all within about 100 beats per minute of the value given by the arithmetic average of the parents of the cross concerned. This is a very close fit indeed, since a fluctuation of 100 beats per minute could easily result from chance factors. This rather exact intermediacy, regardless of whether the mother was from the faster or slower flying strain, is evidence that there is no appreciable non-genetic maternal influence on wing-beat frequency.

The F_1 of the *D. miranda* × *D. pseudoobscura* cross was also intermediate between the parent species, though not so exactly intermediate as in the Race A × Race B cross. The value for this F_1 was not well established for lack of animals. With an adequate number, it might well have been more exactly intermediate. It is unfortunate that the F_1 of the species cross is sterile in both sexes, thus preventing backcrossing. The evidence from the

TABLE 2

The inheritance of wing-beat frequency in D. pseudoobscura. The figures in parentheses are the arithmetical averages of the parental rates.

GENERATION	FREQUENCY OF WING-BEATS (IN THOUSANDS PER MINUTE)
Parental stocks	
Willapa Bay B	10.14±0.07
Perpetua A	11.45±0.05
F_1 from ♀ Perpetua A	10.91±0.04
×♂ Willapa Bay B	(10.80)
Backcross of F_1 ♀	10.61±0.06
×♂ Willapa Bay B	(10.52)
Backcross of F_1 ♀	11.27±0.07
×♂ Perpetua A	(11.18)
The inheritance of wing-beat frequency in the species cross, D. pseudoobscura×D. miranda.	
Parental stocks	
Mt. Whitney (*D. miranda*)	9.95±0.07
Perpetua A (*D. pseudoobscura*)	11.45±0.05
F_1 from ♀ Perpetua A	10.41±0.20
×♂ Mt. Whitney	(10.70)

species cross supports our conclusion that the inheritance of those morphological and other differences which compose the "physiological wing-beat character" is of the multiple factor type.

SEPARATION OF RACES A AND B BY MORPHOLOGICAL CHARACTERS

The only morphological difference between Races A and B, so far reported, is that of the number of teeth in the sex combs referred to previously. This character is not too satisfactory, however, because of overlapping of the number between some localities of the two races. Individuals also vary to such an extent that the right foot may have the number of teeth characteristic of Race A, while the left foot has the number characteristic of Race B, and *vice versa*. In an effort to find other morphological differences which might be less variable, 11 different measurements of the body and its parts were made for all the strains and species considered in this paper. Measurements which include the abdomen are highly variable and probably useless. Measurements of the head, the thorax, and the wings had low coefficients of variability, thus offering themselves as potentially advantageous material. In relation to flight, the wing and thorax measurements should be the most useful. Averages of the different strains making

up the sample for the race or species should be compared so that any differences of a fundamental nature will become more distinct. These measurements are given in table 3. No statistical treatment is necessary to prove that the wings of *D. miranda* are larger than those of Race B or that those of Race B are larger than those of Race A. The wings of *D. athabasca* are very much larger than those of *D. azteca*, though the body dimensions of *D. athabasca* are smaller. It is apparent that within both of the species complexes the forms living in the warmer habitats have the smaller wings as well as the higher wing-beat frequencies. While the thorax of Race B is

TABLE 3

Averages of thorax and wing measurements in the D. pseudoobscura–D. miranda and D. athabasca–D. azteca species complexes.

SPECIES	WING-BEAT FREQUENCY	WING AREA IN SQ. MM.	WING LENGTH	WING WIDTH	THORAX LENGTH	THORAX WIDTH	THORAX DEPTH	NO. OF STRAINS CONTRIBUTING TO THE AVERAGE
D. pseudoobscura								
Race A	10.93	2.44	2.83	1.17	1.24	0.92	0.88	5
F_1 of A×B	10.80	2.56	2.96	1.18	1.28	0.90	0.90	2
Race B	10.46	2.73	2.98	1.23	1.28	0.93	0.90	4
F_1 of ♀ Race A× ♂ *D. miranda*	10.41	2.93	3.09	1.25	1.38	0.94	0.93	1
D. miranda	9.59	3.30	3.29	1.33	1.40	0.98	0.96	3
D. azteca	11.18	2.30	2.82	1.10	1.21	0.88	0.86	2
D. affinis	10.42	2.31	2.80	1.12	1.20	0.87	0.85	2
D. athabasca	9.25	2.72	2.96	1.22	1.19	0.85	0.83	1

somewhat larger than that of Race A, the difference is much less remarkable than that exhibited by the wings. That larger wings are not necessarily correlated with a larger thorax is clearly shown in the *D. athabasca–D. azteca* complex where the abnormally large wings of *D. athabasca* are attached to a thorax distinctly smaller than that of *D. azteca*.

Considering all data, only one correlation remained free of discordance—that between warmer habitat and smaller wings. Furthermore, a relationship emerges which indicates that if body size were held constant, the strain with the smallest wings would have the highest wing-beat frequency.

The correlation between habitat and wing size indicates that wing size might be a satisfactory morphological characteristic by means of which Races A and B could be separated. One empirical method which might succeed would be that of an index number derived as the product of a few measurements of the wing. Such an index number results if the wing area in

TABLE 4

Index number and number of teeth in the sex combs of D. pseudoobscura, D. miranda, and various hybrids.

SPECIES	RACE	INDEX NO.	AV. NO. OF TEETH IN PROXIMAL COMB	AV. NO. OF TEETH IN DISTAL COMB	LOCALITY
	A	52.72	6.11	5.18	Willapa Bay, Wash.
	A	45.72	6.95	5.87	Perpetua, Ore.
	A	56.80	6.72	5.72	Kaibab, Ariz.
	A	60.44	6.51	5.54	Prescott, Ariz.
	A	62.84	6.56	5.31	Quezaltenango, Guatemala
	A Average	55.70	6.57	5.52	
	Hybrids	66.12	6.87	5.25	F_1 of ♀ Willapa Bay B ×♂ Perpetua A
D. pseudoobscura		66.36	6.31	5.61	F_1 of ♀ Perpetua A ×♂ Willapa Bay B
	Hybrid Average	66.24	6.59	5.43	
	B	74.00	6.27	5.63	Willapa Bay, Wash.
	B	76.16	6.58	5.32	Perpetua, Ore.
	B	71.32	5.89	4.64	Yolla Bolly, Calif.
	B	68.80	6.20	4.80	Stony Creek, Calif.
	B Average	72.57	6.24	5.10	
Hybrid, *D. pseudoobscura*× *D. miranda*		85.92	No males result from this cross		F_1 of ♀ Perpetua A ×♂ Mt. Whitney
		130.88	8.04	5.67	Cowichan, B.C.
		108.16	8.94	6.46	Orick, Calif.
D. miranda		114.32	7.31	5.50	Mt. Whitney, Calif.
	Average	117.79	8.10	5.88	

square millimeters is multiplied by the wing length cubed. The reason for using this particular index number will become clear later. The index number for the geographic strains and its average for Races A and B and for the reciprocal F_1 populations as well as *D. miranda* are given in table 4. Here for the first time it may be seen that there is no overlapping between the strains of Race A and those of Race B. Even the F_1 is distinct from any of the strains of Race A or B.

It is true that more strains should be examined before it would be safe to propose this method as one certain of success. However, the measurements of the wing and the calculation of the index number can be done by any technician and upon preserved material. Probably comparison of the index numbers is reliable only when the animals are raised under similar temperature and food conditions.

A count of the teeth of the sex combs of the geographic sample from which the index number was obtained will certainly clinch the determination. We have made counts of the teeth of these strains (table 4) and find that those geographic strains of one race which tend to have the number of teeth found in the other race did not tend likewise to overlap in regard to the index. As a rule if one of the methods is weak, one may expect the other to be stronger and *vice versa;* thus the two methods complement each other. A simultaneous consideration of both methods makes certain, upon purely morphological grounds, the classification of a sample as belonging to Race A or Race B.

THE RELATION OF MORPHOLOGY TO WING-BEAT FREQUENCY

During the flight measurements a general relationship was noted among the various species and strains: low wing-beat frequencies were found in species having a low value of the ratio between the volume of the thorax and the area of the wings. Conversely, higher wing-beat frequencies were associated with higher values of this ratio.

The relationship between wing-beat frequency and wing and thorax size may be examined most readily by assuming that for each species the energy output per stroke is proportional to the volume of the thoracic flight muscles. There is every reason to believe this to be true, since the force exerted by the muscles during a contraction would be related to their total cross-sectional area; whereas the amount of contraction, or distance through which the force acts, would be proportional to their mean length. Hence:

$$\text{Energy per stroke} \propto \text{muscle volume} \qquad (1)$$

Neglecting internal and external energy losses, this stroke-energy is expended in giving a corresponding kinetic energy to the air. Hence, if m represents the mass and v the velocity of the air moved per stroke, then:

$$\tfrac{1}{2}\, mv^2 \propto \text{muscle volume} \qquad (2)$$

But the mass of air moved per stroke is proportional to the product of the wing area and the wing length, for in each stroke the wings sweep out a mass of air whose volume is related to $\pi r^2 h$ (where r represents wing length and h wing width). Equation (2) becomes therefore:

$$\tfrac{1}{2}\pi \text{ (wing area) (wing length) } v^2 \propto \text{muscle volume} \qquad (3)$$

TABLE 5

Morphological characteristics of various Drosophila species and strains. Measurements and their standard errors are given in millimeters.

NO.		STRAIN	WING-BEAT FREQUENCY	WING AREA IN SQ. MM.	WING LENGTH	THORAX LENGTH	THORAX WIDTH	THORAX DEPTH	VOLUME OF THORAX (MUSCLE) (AREA OF WING)(LENGTH OF WING)^2
1		*D. immigrans*	9.97±0.07	3.19±0.05	3.40±0.02	1.52±0.01	1.04±0.01	1.10±0.01	0.0139
2		*D. virilis*	9.37±0.15	3.39±0.05	3.23±0.04	1.45±0.02	1.03±0.01	0.99±0.03	0.0130
3	*D. pseudoobscura*	Willapa Bay A	11.47±0.04	2.31±0.01	2.83±0.01	1.26±0.01	0.90±0.01	0.89±0.01	0.0190
4		Perpetua A	11.45±0.05	2.23±0.02	2.73±0.02	1.21±0.01	0.88±0.01	0.87±0.01	0.0204
5		Kaibab A	10.48±0.10	2.48±0.04	2.84±0.02	1.25±0.01	0.90±0.01	0.86±0.01	0.0170
6		Prescott A	10.81±0.04	2.53±0.03	2.88±0.02	1.22±0.01	0.92±0.01	0.87±0.01	0.0162
7		Quezaltenango A	10.42±0.06	2.63±0.03	2.88±0.02	1.27±0.01	0.98±0.04	0.87±0.01	0.0154
8		Willapa Bay B	10.14±0.07	2.74±0.02	3.00±0.01	1.28±0.01	0.92±0.01	0.90±0.01	0.0143
9		Perpetua B	10.54±0.07	2.82±0.03	3.00±0.02	1.28±0.01	0.95±0.01	0.90±0.01	0.0145
10		Yolla Bolly B	10.43±0.06	2.75±0.03	2.96±0.02	1.23±0.01	0.92±0.01	0.88±0.01	0.0140
11		Stony Creek B	10.72±0.05	2.60±0.02	2.98±0.02	1.31±0.01	0.93±0.01	0.91±0.01	0.0160
12		F_1 ♀ Willapa Bay B ×♂ Perpetua A	10.70±0.11	2.55±0.02	2.96±0.02	1.27±0.01	0.89±0.01	0.90±0.01	0.0155
13		F_1 ♀ Perpetua A ×♂ Willapa Bay B	10.91±0.04	2.56±0.02	2.96±0.02	1.30±0.01	0.90±0.01	0.90±0.01	0.0158
14		F_1 ♀ Perpetua A ×♂ Mt. Whitney	10.41±0.20	2.93±0.03	3.09±0.02	1.38±0.01	0.94±0.01	0.93±0.01	0.0140
15	*D. miranda*	Cowichan	9.27±0.08	3.39±0.02	3.37±0.02	1.36±0.02	0.97±0.01	0.95±0.03	0.0097
16		Orick	9.56±0.06	3.29±0.04	3.21±0.01	1.42±0.01	0.99±0.01	0.95±0.01	0.0122
17		Mt. Whitney	9.95±0.07	3.23±0.03	3.29±0.02	1.42±0.01	0.99±0.01	0.97±0.01	0.0120
18		*D. athabasca*	9.25±0.09	2.72±0.03	2.96±0.02	1.19±0.01	0.85±0.01	0.83±0.03	0.0120
19		*D. azteca* (Deer Creek)	11.26±0.03	2.43±0.01	2.86±0.02	1.26±0.01	0.91±0.01	0.90±0.01	0.0182
20		*D. azteca* (Huehuetenango)	11.09±0.11	2.18±0.04	2.77±0.02	1.16±0.01	0.85±0.01	0.83±0.01	0.0177
21		*D. repleta*	10.62±0.05	3.42±0.04	3.34±0.02	1.56±0.01	1.09±0.01	1.12±0.01	0.0150
22		*D. funebris*	9.62±0.05	3.63±0.06	3.46±0.04	1.50±0.01	0.99±0.01	1.00±0.01	0.0100
23		*D. melanogaster*	10.67±0.05	2.13±0.01	2.61±0.01	1.15±0.01	0.83±0.01	0.81±0.01	0.0204
24		*D. duncani*	10.15±0.10	2.33±0.03	2.82±0.02	1.23±0.01	0.87±0.01	0.85±0.01	0.0175

Furthermore, the velocity imparted to the air (v) is proportional to the product of wing length and wing-beat frequency. Substituting (wing length)^2 (wing frequency)^2 for v^2 in equation (3), we get:

$$\tfrac{1}{2}\pi \text{ (wing area) (wing length)}^3 \text{ (wing frequency)}^2 \propto \text{muscle volume} \quad (4)$$

Transposing and absorbing all constants of proportionality:

$$\text{(wing frequency)}^2 = k \text{ [muscle volume/(wing area)(wing length)}^3\text{]} \quad (5)$$

This relationship may be tested by plotting wing-beat frequency squared on one axis of a grid and the thorax-wing dimension value on the

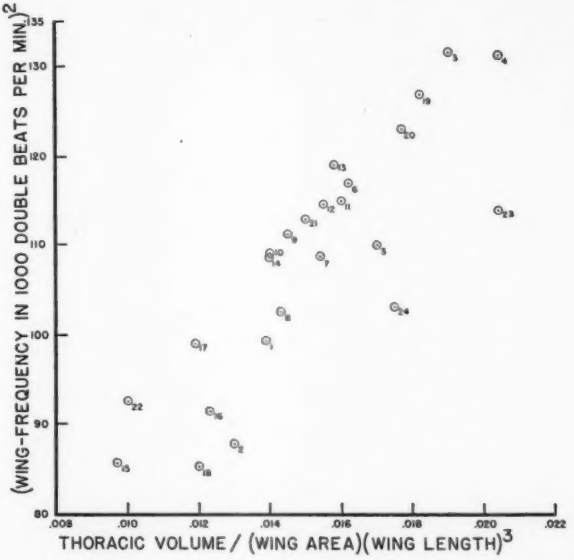

FIGURE 2.—Relation between wing-beat frequency and thorax and wing size for 24 species or varieties of Drosophila. The points may be identified by reference to table 5.

other axis. If the relationship is valid, the points for the 24 different geographic varieties and species should fall into line; if false, the points should be distributed on the grid at random. It proved impractical to measure the muscle volume directly. However, since thoracic muscles occupy, roughly, a constant fraction of the thoracic volume, we were able to use the product of thoracic length, width, and depth as an index of muscle volume.

The basic data necessary for the computations are gathered in table 5, and the plot of these data is the grid of figure 2. The various strains and species agree well with the theoretical thorax-wing dimension relationship. Furthermore the four stocks which fit least well, *D. athabasca*, *D. virilis*, *D*

duncani, and *D. melanogaster*, have lived in laboratory bottles for several years, while the other strains were more recently captured in nature. Perhaps the existence in small bottles, where flight is not essential, has allowed the fixation of genes which upset the relationship. Genes of this kind, which apparently cause a slower wing-beat frequency than the thorax-wing dimensions warrant, would probably be eliminated if natural selection were operative, as it has been with the other strains concerned.

The agreement of the strains is even more remarkable when one considers some of the sources of error which have entered. These errors may be briefly stated as follows:

(1) The actual amount of muscle was not determined, only its index, the volume of the thorax. (2) Possible differences in the morphology of the muscle and in the details of the wing stroke of the various strains were not studied. (3) Though all animals were three days of age, a three day old *D. repleta* is physiologically different from a three day old *D. azteca*. As will be shown elsewhere, there is a relation between physiological age and wing-beat frequency. (4) All animals were flown at 20°C but there may be differences among these species in their response to an identical temperature. (5) Efficiency of the flight system, drag loss, etc., has been neglected. (6) Other, at present intangible, factors such as nutrition, quantity of glycogen present, nervous reflex systems and so on have been ignored.

Numerous errors like these are as likely to be positive as negative in their effects and therefore may be expected to cancel out to a considerable degree. Apparently the errors did cancel to a large extent as evidenced by the correlation coefficient of 0.85 ± 0.05 between the wing-beat frequency squared and the thorax-wing dimensions. This correlation is large and highly significant when compared with its standard error or when tested in Fisher's table of significance for correlations based on relatively few pairs of observations.

A relationship between the thorax-wing dimensions and the wing-beat frequency, which depends upon such basic laws of physics as those concerned with work and air resistance, should be found within other natural groups of insects.

SUMMARY AND CONCLUSIONS

By means of a stroboscopic method the frequency of wing-beat during "fixed" flight was measured for 24 species and varieties of Drosophila. Each species or variety under standard conditions was found to have a characteristic range of wing-beat frequencies. It was thus possible to compare the various strains in terms of this physiological variant.

The average difference in wing-beat frequencies of Race A and Race B of *D. pseudoobscura* is highly significant, amounting to 7.34 times its stand-

ard error. A number of geographic varieties within each race also showed significantly different wing-beat frequencies.

The wing-beat frequencies of an array of geographic strains of *D. pseudoobscura* and *D. miranda* showed no clear correlation with the altitude or latitude from which they were collected. However, the average wing-beat frequencies for Races A and B of *D. pseudoobscura*, *D. miranda*, *D. athabasca*, and *D. azteca* showed a positive correlation with the optimal temperature characteristic of each of these races or species. In general, higher wing-beat frequencies are found for species having higher optimal temperatures.

The inheritance of wing-beat frequency was shown to be of the multiple factor type.

A new morphological method was found for separating Races A and B of *D. pseudoobscura*, entailing the use of index numbers derived from wing measurements.

The differences in wing-beat frequency between species, races, and geographic varieties were found to rest, in large part, upon a striking morphological relationship. This relationship is between the volume of the thoracic flight muscles and the size of the wings. If the muscle volume is held constant, wing-beat frequency increases uniformly as the wing size decreases.

The genetic changes which have separated strains of Drosophila in regard to the "physiological" wing-beat character are therefore gene changes affecting, primarily, the proportional relations between the volume of wing muscle and the wing size.

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